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AUGUST, 1948

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Cohen (p. 445) has investigated oral effects in two individuals with pituitary dwarfism. This rather complete study confirms the observation that the size of the teeth is not affected in pituitary dwarfs and that the crowns develop according to the dwarfs' chronological age. The pulp, roots and supporting structure of the teeth are retarded in pituitary dwarfs even when the patient is given intermittent hormonal treatment. The mandible seems to show greater retarded development than does the maxilla. The teeth, maxilla, and mandible have individual and independent growth patterns which are affected differently in pituitary dwarfism. Because of the lack of synchronization between these structures, there is an interference with position of the teeth in their supporting structures. The observations also confirm the findings that the growth of the ramus length is retarded and the intermaxillary space fails to increase in pituitary dwarfism.

Potter and Meredith (p. 459) have compared two methods of obtaining biparietal and bigonial measurements. While the paper is strictly a discussion of method, it affords some interesting observations. As a result of their work, the authors suggest that in gross studies of young children biparietal diameter be taken by direct measurement and bigonial diameter from measurements on radiographs. The other workers in the field of radiography should be interested in the magnification factor which Potter and Meredith demonstrated as it may have implications in oral surgery, measurements of the temporomandibular joint, and other fields.

Kreshover and Bevelander (p. 467) exposed pulps on three dogs and studied the healing process. The tissues show acute and chronic inflammatory reactions and granulation tissue. The repair process continues to fibrosis and calcified formations are found. Although the authors point out that various anatomical and physiologic factors must be considered, the fact that the pulp has power of repair is demonstrated again.

Belding and Belding (p. 480) discuss the relationship of the oral flora to general body changes. They suggest that modern civilization has introduced an environmental factor which reduces the effectiveness of the antibacterial activity of saliva and that sucrose is probably the most important factor. They use dietary means to eliminate the bacteria and to increase autarcetic immunity. It is most interesting that although the Beldings use a streptococcus as their indicator and others use lactobacilli, the diets used are quite similar.

Hunt and Hoppert (p. 486) at the suggestion of Gies, investigated the correlation between sex and caries in the albino rat. From studies on 853 resistant male and 888 resistant female animals, they conclude that any differences are slight and if femaleness (there is such a word) increases resistance to caries, its influence is slight. Hunt, Hoppert, and their co-workers have utilized their inheritance-caries study rat colony to evaluate the influence of occlusion, age, and sex on rat caries. When good records are kept, such experimental material can be analyzed in many ways. Since the authors suggest that no studies on the effect of "low physical vigor" on caries are available, they might investigate the effect of this factor and of body weight on caries. We, also, have wondered if their caries susceptibility and resistance in rats might not be a result of resistance to fracture. A physical study of the resistance to fracture of teeth from the two groups seems indicated.

Wakeman, Smith, Zepplin, Sarles, and Phillips (p. 489) found that raising the dietary sucrose level from 2 to 10 per cent did not appreciably alter the oral bacterial count nor the caries activity in cotton rats. However, honey in the diet resulted in greater caries activity. Lactobacilli and, in greater numbers, enterococci were isolated from the carious teeth. Whether the same *conditions* are true in man requires further demonstration.

Goldberg, Gilda, and Tishkoff (p. 493), in order to study the amino acids in saliva, utilized the paper partition chromatography method. They were able to identify thirteen free amino acids in the saliva. Previously, Kirch, Kesel, O'Donnell, and Wach had shown the presence of sixteen amino acids in the saliva. The chromatography method also demonstrated the presence of peptides. The authors believe their method has advantage because of the identification of free amino acids without interference of other nitrogenous products. The problem of amino acids in the saliva is important in dental caries research investigation and probably should be considered in studies of other diseases of the oral structures.

Roberts, Bibby, and Wellock (p. 497) had a group of children use an acidulated fluoride mouthwash for one year and found a 30

per cent *higher* caries attack rate in the 187 children using the fluoride by comparison with the 169 not using it. Here is an increase in caries attack while using fluoride. The authors present an hypothesis to explain the possibility. At risk of appearing to labor a point, might we not suggest that increased resistance of powdered enamel to acid decalcification may be totally unrelated to caries resistance? While, in vitro, lead fluoride was more effective in reducing enamel solubility, it was ineffective in reducing caries attack rates (Knutson and Galagan) when applied topically. Now, acidulated fluoride mouthwash appears to increase caries rates. Fortunately, this group tried their method clinically before speaking out for general application. Some other alleged caries inhibitors have never been tested clinically. The test tube is a valuable place to test out ideas and may save years of slow clinical study but, in vivo, results are not always in accord with those obtained in vitro. Both the laboratory and clinic are essential to progress in dental research.

Kitchin and Robinson (p. 501) report the results of a study of the effectiveness of dentifrices of varying abrasiveness in removing the so-called brown pellicle which forms on the teeth of an appreciable percentage of individuals who do not use an abrasive in their dentifrice. The pellicle is not caused by the dentifrice itself but is formed in certain mouths when abrasives are not present in the dentifrice. It was found that beyond a certain degree of abrasiveness, wear on cementum and dentin of exposed roots is greater than necessary. There appears to be no value in increasing the abrasiveness beyond these levels although several commercial dentifrices are more abrasive than the levels suggested. It would seem that continued studies of the abrasiveness of whole dentifrices against the cementum and dentin of teeth would be a worth while project for some agency of organized dentistry so that the dental profession might be advised an nually of the degree of abrasiveness to which teeth of their patients are subjected.

Hinkel, Nachod, and Tainter (p. 507) describe a method for particle size determination and give the results of studies on standard powders such as are used in dentifrices. Since dentifrice abrasion as well as its polishing ability and ability to keep teeth free of brown pellicle may be in large part a function of particle size, such determinations are useful.

Dawson (p. 512) took advantage of opportunities and examined the oral cavities of 994 Egyptian agricultural workers in a hospital. He found fewer cavities per mouth than in countries with a higher standard of living. He explains this condition, in part, on the basis of high mineral content and high fluoride content (.71 ppm) of the water. One possibility that was not discussed was the possibility of a lower caries rate among those hospitalized Egyptians than among their healthier fellows. Mann, Driezen, Spies, and Hunt (*J. A. D. A.* 15: 244, 1947) have demonstrated 30 per cent of the number of cavities in malnourished patients compared with regional averages. The high incidence of periodontal disease might be expected in an area of malnutrition and oral neglect.

Jensen and Möller (p. 524) have studied specimens of human enamel and dentin and of rabbit and beaver enamel for their apatite particle size and shape. The found great differences in the human incisors, their approximately twice the size (600A°) of specimens from a human molar (250 to 300A°). Variations between particle form from enamel and bone were observed. These studies "beyond the microscope" may offer valuable information when correlated with conventional and electronic micoscopic observations.

Yardeni (p. 532), presents an interesting study of the possible antibacterial activity of calculus. He found that calculus favored the establishment of gram-negative bacilli in vitro and that proteolytic bacteria were almost absent in the zone about calculus flakes. Calculus also was demonstrated to inhibit germination of wheat grains. Yardeni is not advocating leaving calculus on teeth, recognizing its importance as an etiological factor in gingivitis, but suggests that the caries-calculus relationship is worthy of further study.

Shulman and Robinson (p. 541) present the results of salivary citrate analyses of twenty-five individuals without erosion and fifteen with gross erosion. No correlation between the presence of erosion and salivary citrate was noted, but it was not ascertained whether or not the process of erosion was *active* at the times the specimens were collected. This is similar to many apparently well-planned studies in which an uncontrolled factor is found that was overlooked. A useful finding is the occurence of erosion in 2 per cent of 1,345 young men.

H. B. G. R.

DENTAL DEVELOPMENT IN PITUITARY DWARFISM

M. MICHAEL COHEN, D.M.D., AND RICHARD WAGNER, M.D.

From the Department of Pediatrics, Tufts College Medical School, the Boston Floating Hospital, and the Children's Department, Boston Dispensary, Boston, Mass.

TRUE pituitary dwarfism manifests itself clinically in general retardation of skeletal development and in hypogonadism. Other findings occasionally associated with hypopituitarism are obesity, geroderma, and diabetes insipidus, although these are not necessary for the diagnosis of pituitary dwarfism.[1] The deficiency in pituitary dwarfism can result from congenital absence of the acidophilic cells in the anterior lobe or surgical removal, pressure necrosis, infarction, calcification, destructive lesions, or tumors in or adjacent to the gland.

CASE REPORTS

CASE 1.—W. T., white, male child, born Aug. 21, 1939 (Fig. 1).

This patient was first hospitalized at the age of 2 years, nine months because of polydipsia and polyuria from the age of 1 year. His family history was noncontributory and his environment was good. Prenatal diet had been adequate, the labor prolonged and forceps had been used. His birth weight was 7½ pounds. He was breast fed for six months, then received lactogen until the age of 1 year. His nutritional history was adequate and accessory vitamins had been given. Growth and development had been retarded, but the patient appeared mentally normal. He had measles at the age of 1 year, mumps at 2½ years, and upper respiratory infection and otitis media three months prior to admission.

The onset of the present illness became apparent at the age of 1 year when excessive thirst and frequency of urination developed. The patient was said to drink a gallon of water daily and to wake up several times nightly because of thirst. The frequency of urination was marked, occurring every fifteen minutes during the day and four times during the night. There was some discomfort after voiding. There was no polyphagia.

Physical examination revealed a child mentally alert and hyperirritable who appeared to be about 1 year old. The temperature was 99.4° F., respiration 20, and pulse 120. The skin was warm and moist, the hair was silky, the fingernails were smooth and glistening, and the tonsils slightly enlarged. There was a systolic murmur over the tricuspid area. The abdomen was somewhat protuberant and the urinary bladder was felt just below the umbilicus. Dribbling of urine took place when the patient cried. The lungs were clear; rectal examination was negative. Both testicles were in the scrotal sac but were migratory and each was the size of a small pea and of very soft consistency. The reflexes were active. His height was 29¾ in., and he weighed 23 pounds.

Repeated urinalyses were negative; the specific gravity varied from 1.003 to 1.010. The blood examination showed slight hypochromic anemia. Hinton, Wassermann, and Kahn tests were negative. The tuberculin test (1 mg. intracutaneously) was negative. No attempt at concentration of urine was made. The blood cholesterol was normal.

Radiographs of the skeleton showed a small sella turcica, and ossification of the carpal bones was consistent with that of a child 1 year of age (Fig. 2). The glucose tolerance test was normal. The patient had polydipsia throughout his hospital stay, taking from 6 to 56 oz. of fluid daily. His growth and treatment chart is shown in Fig. 3.

Diagnosis: Pituitary dwarfism.

Presented at the Twenty-Fifth General Meeting of the International Association for Dental Research, Chicago, Ill., June 21-22, 1947. (*J. D. Res.* 26: 468, 1947.)

Received for publication Feb. 10, 1948.

Fig. 1.—Case 1. Photographs of the patient at the age of 2 years, 9 months, and at 7 years, 6 months.

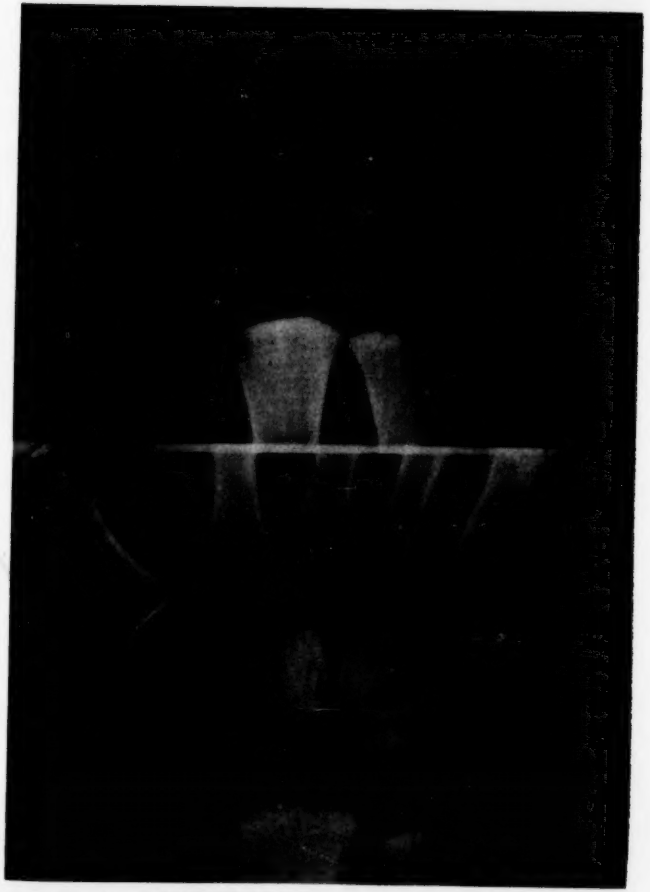

Fig. 2.—Radiograph of wrist of patient (Case 1) at age of 2 years, 9 months (above) as compared with that of a normal child the same age (below).

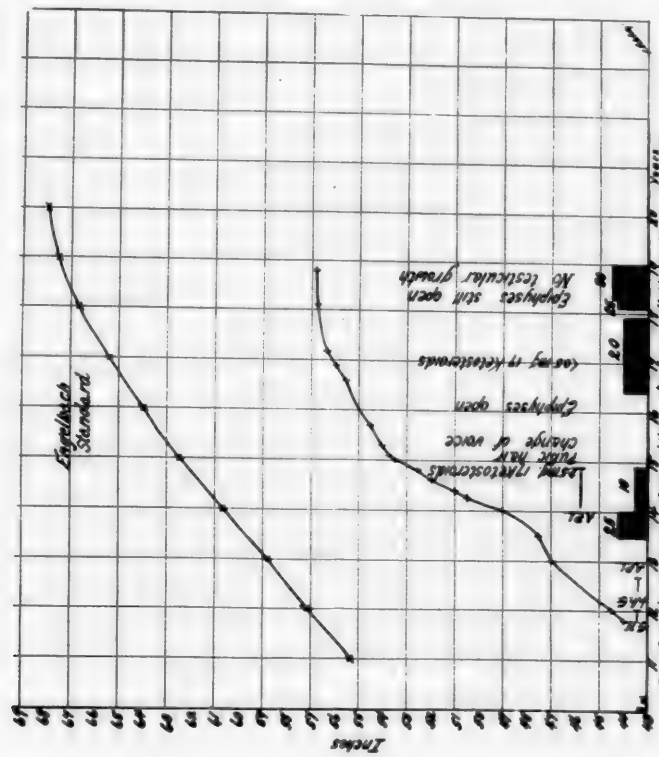

Fig. 4.—Case 2. Growth curve and treatment chart of patient.

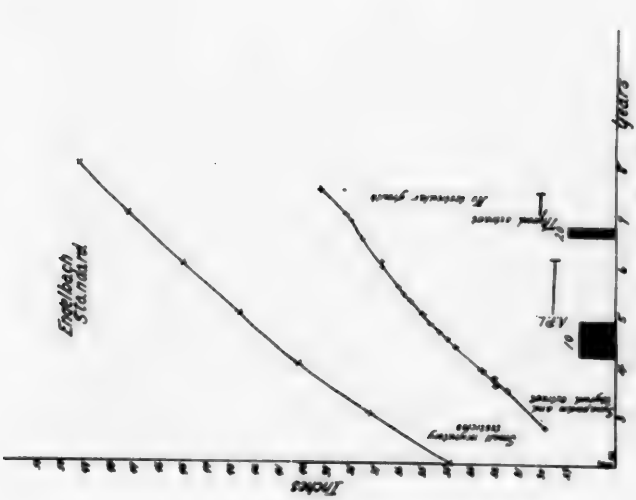

Fig. 3.—Case 1. Growth and treatment chart of patient.

CASE 2.—L. M., white, male child, born March 8, 1928.

L. M. was first seen at the Children's Hospital Out-Patient Department in February, 1929, at the age of 11 months because of failure to gain weight after an attack of pertussis. Simple adjustment of feeding was made at that time. He was next seen in April, 1933, at the age of 5 years because he was not growing as rapidly as a normal child. Physical examination revealed an undersized but normally proportioned child. Radiographs of the wrists showed an osseous development corresponding to that of a 2-year-old child. His basal metabolic rate was –14 and his I. Q. was 85 at that time.

L. M. was then seen at the Children's Clinic of the Beth Israel Hospital at the age of 11½ years because of retarded physical development. Radiographs of the upper and lower extremities showed marked retardation of osseous development. The bone age was consistent with that of a 5- to 6-year-old child. He was 44 in. tall. There was evidence of retardation in dental development. The basal metabolic rate now was –10. The glucose tolerance test showed: fasting, 122 mg. per cent; half-hour, 219; one hour, 128; and three hours, 98 mg. per cent. A slight degree of obesity was noted at this time. Both testicles were descended and were about the size of cherry stones but of mushy consistency.

Diagnosis: Pituitary dwarfism (Figs. 4 and 5).

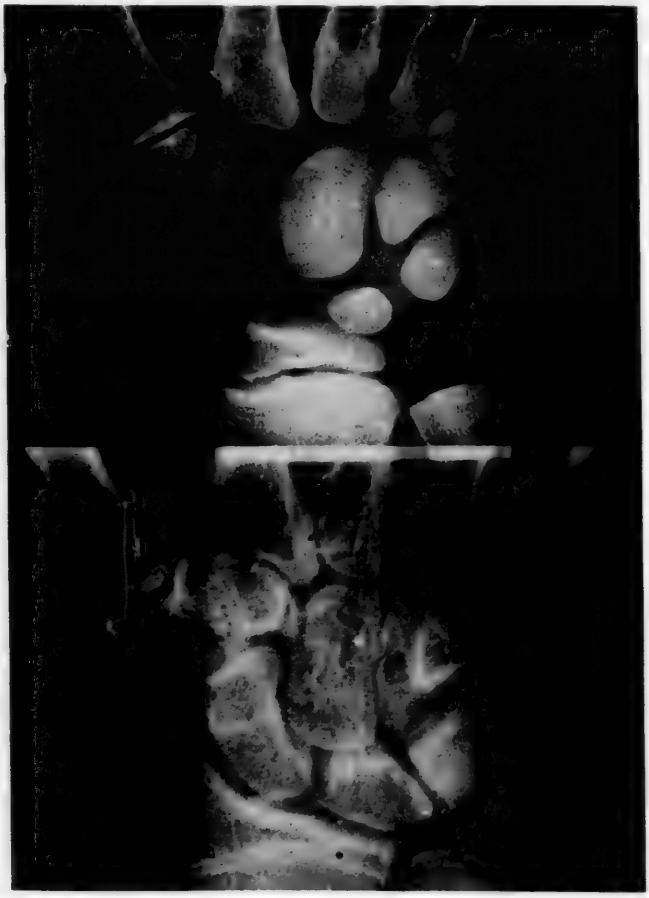

Fig. 5.—Case 2. Radiograph of wrist of patient at age of 13 years (above) as compared with that of a normal 13-year-old boy (below).

These two cases are of interest because they represent the same form of dwarfism at different ages. Both revealed the two essential features of pituitary dwarfism, i.e., stunted growth and failure of gonadal development. They were similar in external appearance. Radiographs of both patients showed marked retarded osseous development. The bio-assay in the older dwarf revealed a 17 ketosteroid excretion of less than 0.5 mg. at the age of seventeen years. Other forms of dwarfism can be ruled out, although the diagnosis of pituitary dwarfism is sometimes difficult before pubescence because of the undeveloped genitalia in normal individuals in early childhood. However, the testes in the younger dwarf were smaller than those seen in children of the same age. They were migratory and of poor consistency. Any other manifestation of retarded osseous bone development might be contributory to the diagnosis.

OBSERVATIONS OF DENTAL DEVELOPMENT

In the diagnosis of pituitary dwarfism it would be helpful if other manifestations of retarded osseous development could be demonstrated. It has been possible in each of these cases to observe the development of teeth and jaws over a period of approximately five years: from the ages of 2 years, 9 months to 7 years, 6 months; and from 15 years, 5 months to 19 years, 1 month. Intermittent treatment with growth-stimulating hormones was given. The growth

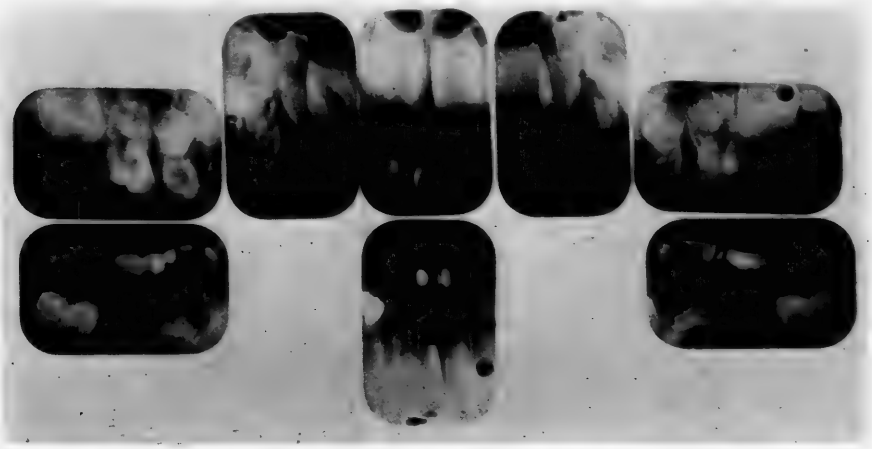

Fig. 6.—Case 1. Intraoral radiographs of patient at age of 6 years, 5 months.

of the developing teeth and jaws was studied clinically and radiographically during this period and impressions of the jaws were taken annually to observe the size of the teeth, the arch growth, the increase in size of the jaws, and the occlusal relation of the teeth. In the younger of the two dwarfs, four mandibular incisor teeth were extracted at the age of 6 years, 6 months. These teeth were prepared for microscopic examination.* The ground sections showed normal enamel, dentin, and cementum. Celloidin sections showed a normal pulp.

*At the Research Laboratory of the Harvard School of Dental Medicine.

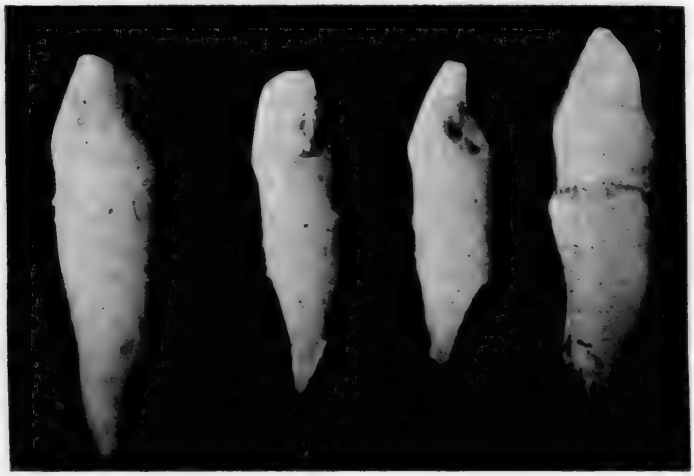

Fig. 7.—Case 1. Extracted mandibular incisors of patient at age of 6 years, 6 months.

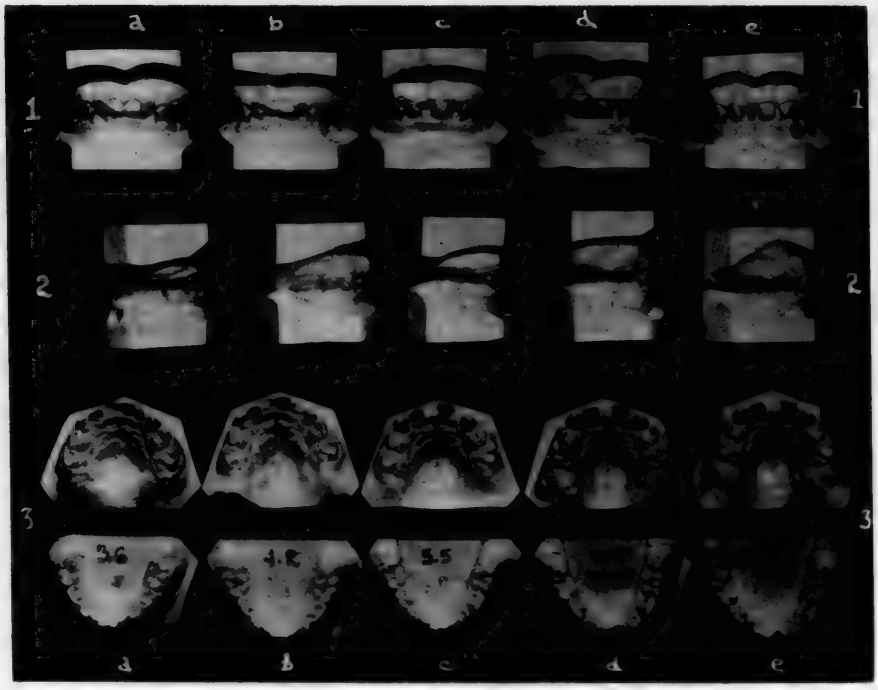

Fig. 8.—Case 1. Maxillary and mandibular casts of patient: *a*, 3 years, 6 months; *b*, 4 years, 2 months; *c*, 5 years, 5 months; *d*, 6 years, 5 months; and *e*, 7 years, 5 months.

At 3 years, 9 months, the individual of Case 1 had a full complement of deciduous teeth. Although the carpal development was that of a child 9 months old, radiographs revealed that the crowns of the permanent teeth were developing according to the patient's chronological age and that the size and morphology of the teeth compared favorably with those of a normal child. Repeated radiographs showed delayed resorption in the deciduous teeth and a marked retarda-

Fig. 9.—Case 2. Maxillary and mandibular casts of patient from the ages of 15 years, 9 months, to 19 years, 1 month: *a*, 15 years, 9 months; *b*, 16 years, 9 months; *c*, 18 years, 1 month; and *d*, 19 years, 1 month.

tion in the developing roots of the permanent teeth. There was also a lack of axial eruption of permanent teeth. Clinical observations of the mouth showed a high caries-attack rate of the deciduous teeth with early pulp involvement and loss of several teeth because of periapical infection.

At the age of 6 years, 3 months, the mandibular deciduous incisor roots showed very little resorption (Fig. 6), and the permanent incisors showed arrested axial eruption. It was decided at this time to extract the four mandibular deciduous incisors (Fig. 7) in the hope of facilitating the eruption of the permanent teeth, but it was not until one year had elapsed that the permanent

mandibular first incisor crowns erupted into the oral cavity almost filling the space formerly occupied by the four deciduous mandibular incisors. Although the maxilla developed posteriorly, the first permanent molars in both jaws had not yet erupted.

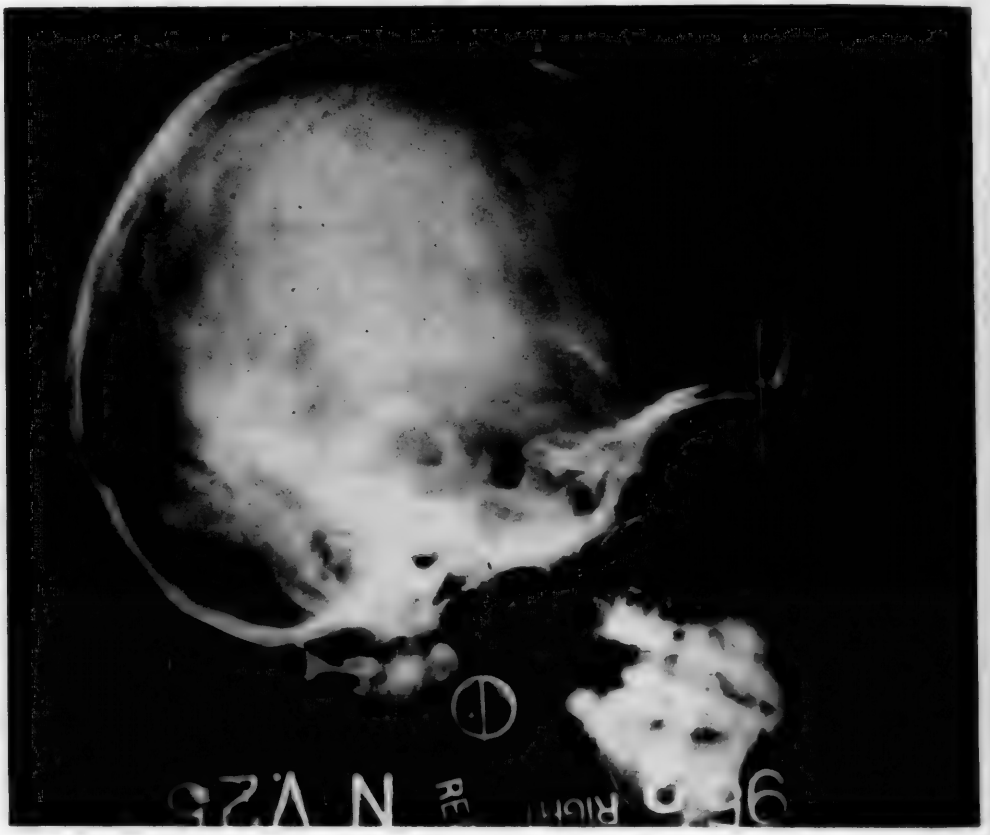

Fig. 10.—Case 2. Lateral skull radiograph of patient at age of 13 years.

Impressions of the jaws, made annually from the ages of 3 years, 6 months to 7 years, 1 month (Fig. 8), provided an opportunity to measure the annual growth and development of the dental arches and the occlusal relationship of teeth and jaws. The growth of the arches was measured by a modified method suggested by Braun[2] and Cohen.[3] It became apparent that in one patient (Case 1) very little growth occurred in the canine region of the maxilla and none in this region of the mandible. This is contrary to the findings in normal children where the greatest lateral growth occurs in the canine region between the fifth and eighth year of life.

The boy discussed in Case 2 (Fig. 9) showed no detectable growth in the dental arches. The teeth which were undererupted at 15 years, 9 months con-

tinued to erupt slowly and the maxillary incisors assumed a marked overbite relationship. This boy had a thorough dental examination at the age of 15 years, 6 months. Prior to this time, repeated references had been made in his medical reports regarding his retarded dental development (Fig. 10). At the age of 15 years, 6 months, the patient had the following maxillary teeth: 7-6-v-4-3-2-1-1-2-3-4-v-6-7. These teeth were undererupted and the arch curve was well rounded with crowding of the incisor teeth. The size of the teeth compared favorably with those seen in normal children. Two deciduous second molars remained in the maxilla and the right second permanent molar had just erupted. The jaw seemed to accommodate all the teeth easily. Lateral jaw and intraoral radiographs showed that the roots of all the erupted permanent teeth were well formed and closed (Figs. 11 and 12).

Fig. 11.—Case 2. Lateral jaw radiographs of patient at age of 15 years, 6 months.

In the mandible all permanent teeth except the second and third molars were present. The size and morphology of all these teeth also compared favorably with those seen in normal children. The anterior portion of the mandible was underdeveloped with crowding of the incisor teeth. The mandible had not developed posteriorly and there was insufficient room for eruption of the second permanent molars. Lateral jaw and intraoral radiographs revealed unerupted second and third molar teeth (Figs. 11 and 12). The roots of the second premolars were not fully closed and the roots of the second permanent molars were still open. At the age of 15 years, 6 months, when the teeth were in occlusion,

the intermaxillary space was negligible; and from 15½ years to 19 years, one month, as the mandible developed posteriorly, the second permanent molar was released into this space. The ramus length developed very slowly and, as it did, the second molars were accommodated into occlusion with no intermaxillary space posterior to them. The maxillary incisors continued to erupt causing a marked overbite condition which further retarded the growth and development of the already underdeveloped mandible.

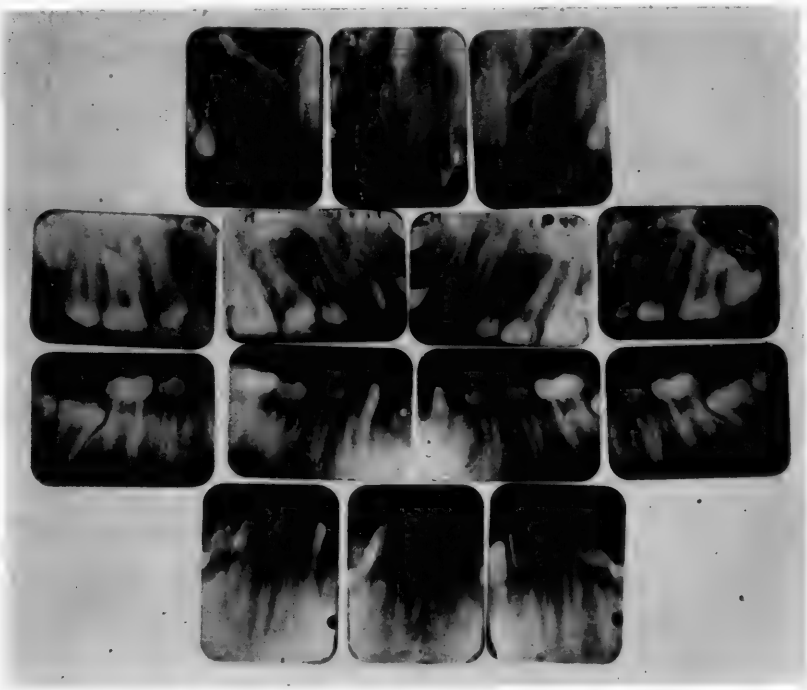

Fig. 12.—Case 2. Intraoral radiographs of patient at age of 15 years, 6 months.

DISCUSSION

Repeated reports[4-9] in the literature suggest retardation of dental development in dwarfism. The most recent was that of Dupertuis[10] who examined a group of three different types of white dwarfs (fetal-like midget, true midget, and miniature) to evaluate them anthropometrically. Although their dental examinations were superficial, enough data were collected to indicate delayed shedding of deciduous teeth and considerable retardation in eruption of permanent teeth. Many of these dwarfs had crowded teeth because of insufficient room in the jaws to accommodate them. It became apparent from these observations and our data that the teeth in pituitary dwarfs are not undersized. This agrees with the findings of Diamond[11] that teeth develop independently of the jaws. Our findings further suggest that the growth of the maxilla and mandible

are not affected in the same degree in pituitary dwarfism. The mandible, like the long bones, is arrested in its growth and development to a much greater degree than the maxilla. This seems to correlate with observations by other investigators[12-15] that there are genetic determiners; one for the maxilla, one for the mandible, and one for the teeth.

In Case 1, with the opportunity of observing the developing permanent dentition of the patient, it became apparent that the crowns of the permanent teeth, the enamel of which is derived from the ectoderm, are not affected in hypopituitarism and develop in accordance with the patient's chronological age (Figs. 13 and 14). However, the pulp and roots of these teeth are derived from the

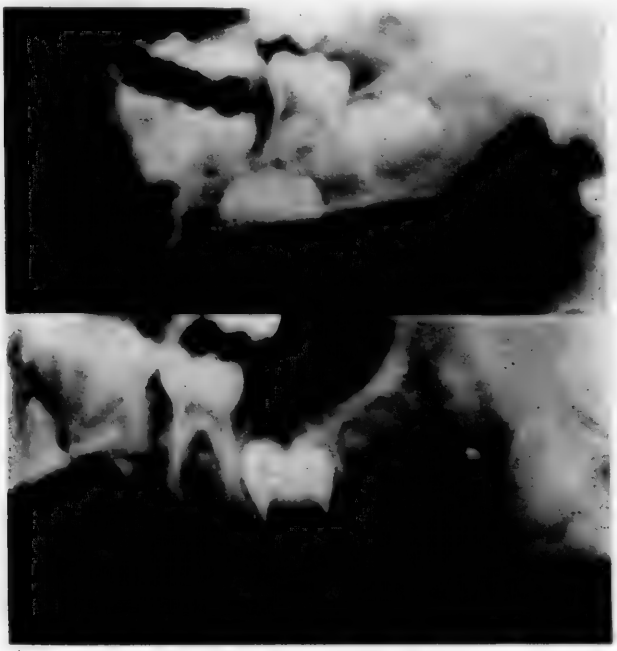

Fig. 13.—Case 1. Lateral jaw radiograph of patient at age of 3 years (above) compared with lateral jaw of normal child same age (below).

mesoderm and are affected, causing a marked retardation in root development. This is contrary to the findings in ectodermal dysplasia[16, 17] where very often teeth are missing and the morphology of the crowns is affected, but the roots of the teeth develop normally, as do the jaws. The osseous development in the maxilla of pituitary dwarfs is not as greatly retarded as in the mandible. Therefore, the developing roots of the permanent teeth in the maxilla are formed earlier, as demonstrated radiographically in Case 2.

The growth of bone in the maxilla and mandible is one of the forces of eruption. This force and proliferation of the pulpal connective tissue motivates the tooth for axial movement, the pressure being directed toward the surface

of the jaws by the presence of a hammock ligament upon which the growing end of the root rests. This hammock ligament is anchored in the bone of the crypt of the tooth and consists of collagenous fibers between which are formed great quantities of fluid which helps in the eruption of teeth.[18-21] All these tissues which are derived from the mesoderm are affected in pituitary dwarfism; this probably accounts for the late eruption of permanent teeth and retardation in the resorption of deciduous teeth.

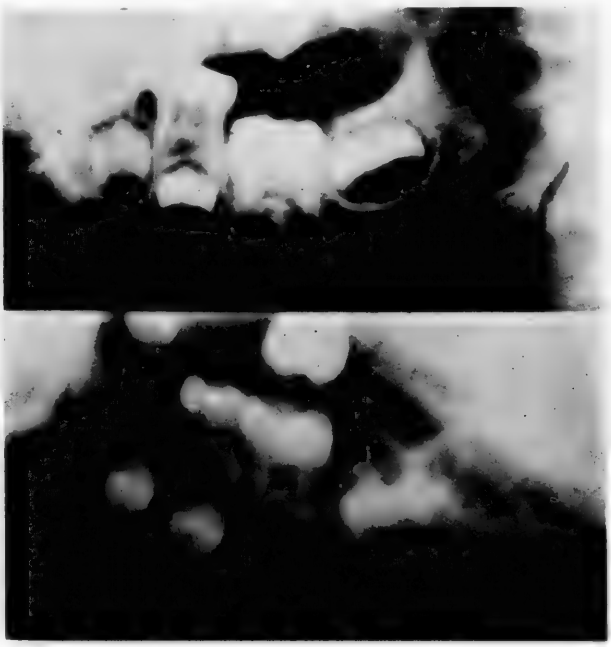

Fig. 14.—Case 1. Lateral jaw radiograph of patient at age of 7 years (above) compared with lateral jaw of normal child same age (below).

In other forms of dwarfism, the dental development is affected in a different manner. We have had an opportunity to see a 12-year-old girl with congenital syphilis whose mental and physical development was markedly retarded. She showed a small maxilla with all the permanent teeth unerupted. The permanent first incisors were typically Hutchinsonian and the roots of these teeth were not well developed. The mandible was also small and had four permanent Hutchinsonian (screw driver-shaped) incisors erupted, but the roots of these teeth were closed. In dwarfism associated with glycogen storage disease, neither the jaws nor the teeth are affected.

It became apparent in the older pituitary dwarf that the retardation of bone growth in the jaws and lack of synchronization with tooth development affected the positional arrangement of the maxillary and mandibular incisors. We may expect to see an interference in the positional arrangement of the permanent teeth in the younger dwarf since he has large permanent teeth.

In both cases the intermaxillary space has failed to increase to any appreciable degree. In the younger dwarf, at the age of 3 years, 6 months, there was practically no intermaxillary space posterior to the second deciduous molar, and even when the patient was 7 years, 5 months, this space failed to develop. In the older dwarf, at 15 years, 6 months, there was very little intermaxillary space posterior to the first permanent molar. This space developed so slowly that not until the age of 19 years, 1 month, was enough room created to accommodate the eruption of the second permanent molars. However, after this had occurred, no intermaxillary space existed posterior to the second permanent molars. These observations demonstrate clearly and confirm the findings of Diamond[22] that in pituitary dwarfism the growth of the ramus length is retarded and the intermaxillary space fails to increase in any appreciable degree. The maxillary incisors continue to erupt causing a marked overbite which inhibits the growth of the already retarded anterior portion of the mandible.

CONCLUSION

The following observations of dental development were made on two pituitary dwarfs who were observed from the ages of 2 years, 9 months, to 7 years, 5 months, and from 15 years, 5 months, to 19 years, 1 month:

1. The size of the teeth in pituitary dwarfs is not affected by the disease.

2. The crowns, the enamel of which is derived from the ectoderm, develop according to the dwarf's chronological age.

3. The pulp, roots, and supporting structures of the teeth, which are derived from the mesoderm, are retarded in pituitary dwarfs, even though the patients are given intermittent hormonal treatment.

4. The osseous development in the maxilla in pituitary dwarfism is not as greatly retarded as in the mandible.

5. The maxilla, mandible, and teeth have their individual and independent growth patterns and are affected differently in pituitary dwarfism. Because of lack of synchronization and interference with normal growth and development of these structures, retarded eruption of teeth takes place as well as interference in positional arrangement of these organs.

6. The lack of ramus length growth in pituitary dwarfism causes inhibited growth of the intermaxillary space and a failure of increase of the vertical dimension of the maxilla and body of the mandible. The maxillary incisors continue to erupt causing a marked overbite relationship and inhibiting the already retarded forward growth of the anterior portion of the mandible.

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A COMPARISON OF TWO METHODS OF OBTAINING BIPARIETAL AND BIGONIAL MEASUREMENTS

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THIS paper pertains to methodology, to that sector of anthropometry known as cephalometric technic. It presents a study of the relative adequacy of cephalometric data collected by alternative procedures.

Two procedures or methods were investigated: (1) a radiographic procedure, and (2) a method of direct measurement. Both were applied in determining selected dimensions of the calvaria and face. The purpose was to compare and evaluate the methods from the standpoints of data reliability and validity.

SUBJECTS

The subjects were ninety-four white children within the postnatal age limits of 4 and 5 years. Fifty were boys and forty-four were girls. All resided in or near Iowa City, Iowa, and were enrolled in the Facial Growth Study (a long-term research program begun at the State University of Iowa early in 1946).* Information obtained on the parents showed them to be almost entirely (97 per cent) of north European ancestry, and predominantly of above average socioeconomic status: 42 per cent of the fathers were in the professional group, and 55 per cent in the managerial, commercial, or skilled-trade groups.

Although the Facial Growth Study is housed with the College of Dentistry, invitation of subjects to voluntary participation is in no way related to their dental conditions or orthodontic needs. A major objective of the program is that of studying individual patterns of growth both in children morphologically normal and in children orthodontically deviant.

Each of the subjects came to the Study examination rooms by appointment arranged by Mrs. Helen Bradley.

DATA

The data utilized for this study were collected during 1946 and 1947. They consisted of records for one calvarial dimension (biparietal diameter) and one facial dimension (bigonial diameter). In each instance, parallel series of records were obtained by two methods.

Method I.—A posteroanterior roentgenogram was secured on each of the ninety-four subjects. These exposures were made by Dr. L. B. Higley after the subjects were carefully oriented in the Higley Head Positioner.† The objective in orientation was to position the child's head in such a way that the x-rays were centered to the intersection of the median plane and the Frankfort Horizontal. A constant distance of 152 cm. (60 inches) was maintained between

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the x-ray source and the plane of the child's auditory meatuses. The cassette was placed at right angles to the target, and in contact with the arm of the positioner supporting the child's forehead.

Each roentgenogram was measured by two trained anthropometrists (J. W. P. and R. D. D.) working independently. Measurement was made of (a) the maximum horizontal breadth of the skull shadow, and (b) the greatest horizontal breadth of the mandibular shadow at the gonion level. Sliding calipers were used.

Method II.—Direct measurements of maximum head breadth and bigonial diameter were also taken for each of the ninety-four subjects. These were determined with spreading calipers. Again, separate records were obtained by two trained anthropometrists (H. B. and H. V. M.).

In taking head breadth, the ends of the calipers were placed against the sides of the child's head and moved around in a horizontal plane until the greatest biparietal diameter was found. Care was taken to remove any obstacles (e.g., hair ribbons, braids, clasps) and to crush or separate the hair in the region measured.

In determination of bigonial diameter, it was a requisite condition that the lateral muscles of the face were relaxed. Further, at the time of firm application of the instrument, special caution was taken to avoid any slipping of either end of the calipers to a site more medial than the lateralmost projection of the mandible at the gonial angle.

FINDINGS FOR BIPARIETAL DIAMETER

Reliability of Measurements.—Reliability of biparietal diameter as determined by each method was studied in two ways: by computing reliability coefficients and by making distributions of difference values. The reliability coefficient obtained from the roentgenographic measurements of J. W. P. and R. D. D., using the Pearson product-moment method of correlation, was .990. An almost identical degree of agreement was found between the direct measurements of H. B. and H. V. M. ($r = .989$). Reliability coefficients of this magnitude indicate extremely high consistency. In other words, for both of the procedures employed, the corresponding records of different anthropometrists tend either to coincide or to approximate each other closely.

The second method of studying reliability was that of making distributions of the absolute (millimeter) variations in the measurements of paired anthropometrists. For each of the ninety-four subjects, the difference was computed (without regard to sign) between the roentgenographic measurement of J. W. P. and that of R. D. D. A similar series of difference values was derived from the direct measurements of H. B. and H. V. M. The tabulation which follows summarizes these materials.

DIFFERENCE INTERVAL (MM.)	X-RAY METHOD		DIRECT METHOD	
	N	%	N	%
0 to 0.9	90	96	80	85
1 to 1.9	4	4	13	14
2 to 2.5			1	1

There is a tendency (also reflected in the reliability coefficients) for the roentgenographic measurements to be slightly more reliable than the direct measurements. The tendency is very slight.

It is pertinent at this juncture to take cognizance of the fact that the foregoing analyses for the radiographic method fall somewhat short of constituting a full study of reliability. Since J. W. P. and R. D. D. measured the same series of roentgenograms (only one exposure for each subject having been made), no account was taken of the unreliability introduced in making the transfer from subject to roentgenogram. Considering the control features and precise calibrations of the head positioner, together with the rigorous standardization of other aspects of the radiographic process, one would expect the variance of skull breadth shadow in repeated exposures to be quite small. This expectation was confirmed. On each of six subjects, two strictly independent roentgenograms, i.e., roentgenograms from completely separate orientations of the subject, were obtained. The breadth of the skull shadow in each roentgenogram was determined by averaging fifteen separate measurements taken by three anthropometrists. The differences found between the six pairs of roentgenograms varied from 0.1 to 0.3 millimeter.

One further consideration is relevant. It is practicable in making anthropometric examinations to augment the reliability of direct measurements of head breadth. This is being done routinely in the Facial Growth Study through the following procedure: After two anthropometrists have each measured head breadth, if their results differ by more than 1 mm. they take additional measurements. The manner in which these two or four records are combined will be discussed later.

Summarizing for reliability, biparietal diameter can be measured on children of preschool age with marked consistency using either the radiographic method or the direct method. Given trained personnel and adequate equipment, both methods are about equally reliable. Employing either method, two trained anthropometrists working independently can obtain records which differ by no more than 1 mm. on over 80 per cent of the subjects measured.

Comparability and Validity of Methods.—How do the values one obtains from direct measurement of head breadth compare with the values secured from measurement of breadth of the skull shadow on the roentgenogram? In other words, are the head-size findings from the two methods equivalent or dissimilar?

For study of this problem it was necessary to have single (and highly dependable) values for each subject and method. Values satisfying these criteria were derived by averaging the separate records on a subject taken by the paired anthropometrists. By calculating the mean of the records of J. W. P. and R. D. D. on each subject, ninety-four values were obtained representing the roentgenographic method. Ninety-four comparable values for the direct method were secured by averaging the records on each subject taken by H. B. and H. V. M. The specific procedure here was that of determining the mean of two records where these did not differ by more than 1 mm., and the mean of four records wherever differences between the first two exceeded 1 millimeter.

The two series of ninety-four values, portraying the same sample of children studied by different methods, were analyzed for central tendency and variability. Results, expressed in millimeters, are presented in the following tabulation.

METHOD	N	MEAN	STANDARD ERROR OF MEAN	STANDARD DEVIATION	STANDARD ERROR OF SIGMA
Direct	94	138.3	±0.6	5.8	±0.4
X-ray	94	145.8	±0.7	6.6	±0.5

Examination of these results shows:

1. Direct measurements of head breadth are systematically smaller than measurements of skull breadth taken on posteroanterior roentgenograms. Specifically, the mean of the values representing the x-ray method exceeds that for the direct method by 7.5 millimeters.

2. Direct measurements of head breadth tend to be less variable than measurements of skull breadth determined on the roentgenograms. It will be seen that the standard deviation is 0.8 mm. smaller for the direct method than for the x-ray method.

What interpretation should be placed on these findings? On the assumption that both methods give biologically valid results for head size, one would expect the x-ray method to yield values slightly smaller, and no greater in variability, than those for the direct method. This follows since on the roentgenogram one is taking a skeletal breadth, while directly on the child one is also including some thickness of integument.*

The findings obtained suggest the hypothesis that two artifacts are operative in the x-ray method: (1) magnification, and (2) differential distortion. Simple magnification would account for the significantly larger x-ray mean, and differential distortion would explain the greater variability.

Magnification was studied on a child's skull made available through the courtesy of the Department of Anatomy, State University of Iowa. By averaging the independent records of three anthropometrists, the direct method of skull breadth determination gave a value of 123.0 millimeters. Marks were made on the parietal bones indicating where this breadth was obtained. Small pins were then attached to the skull at the sites of the marks, and the skull oriented in the head positioner. Two roentgenographic exposures were made, one posteroanterior (Fig. 1) and the other anteroposterior. In each instance, the distance was measured from the cassette to the pins. Both roentgenograms were carefully measured by three different anthropometrists. Findings were:

ROENTGENOGRAM	SKULL BREADTH (MM.)	OBJECT-CASSETTE DISTANCE (MM.)
Posteroanterior	133.6	113
Anteroposterior	129.7	74

*It is possible that part of the expected difference due to thickness of the overlying soft tissues is reduced by orthographic superimposition. If the most lateral extension of a child's head were more anteriorly situated on one side than on the other, the horizontally projected skull breadth would exceed the skull breadth for any vertical plane.

It follows that the divergence of x-rays from their central source produced magnifications in the breadth of this skull of approximately 6.7 mm. at a pin-film distance of about 74 mm., and of 10.6 mm. at a pin-film distance of 113 millimeters.

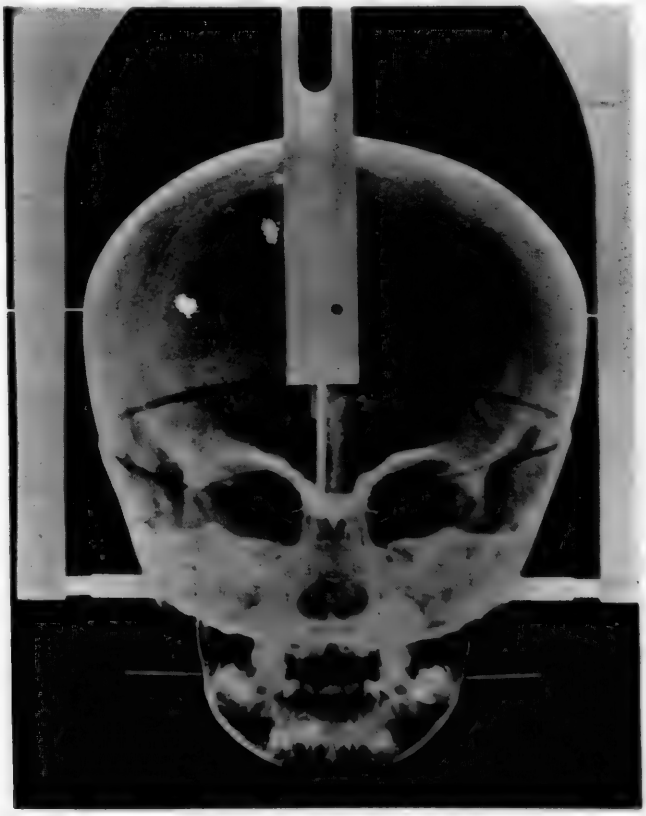

Fig. 1.—Photographic reproduction of a posteroanterior roentgenogram. Attention is called (a) to orientation of child's skull in Higley Head Positioner and (b) to pins attached at parietal and gonial landmarks.

The factor of differential distortion was investigated by comparing subjects of appreciably different head breadths. Utilizing the distribution of values from direct measurement, two subgroups were formed. One group included subjects with head breadths 145 mm. or larger; the other group, those with head breadths 132 mm. or smaller. For each of the subjects in these "broad" and "narrow" subgroups, the difference was obtained between the value by direct measurement and the roentgenographic value. Results from analysis of the two series of differences are displayed in the following tabulation.

HEAD BREADTH SUBGROUP	NUMBER OF SUBJECTS	MEAN OF DIFFERENCES (X RAY MINUS DIRECT)
Broad (145 mm. or more)	12	8.8 mm.
Narrow (132 mm. or less)	15	6.8 mm.

It will be seen that the mean difference was 2 mm. greater for the upper segment of the head breadth distribution than for the lower segment. Generalizing, the broader the child's head, the more its true breadth is exaggerated in the roentgenogram (Fig. 2).

The findings for biparietal diameter may be epitomized as follows:

1. Highly reliable records were obtained by both of the methodological procedures studied.

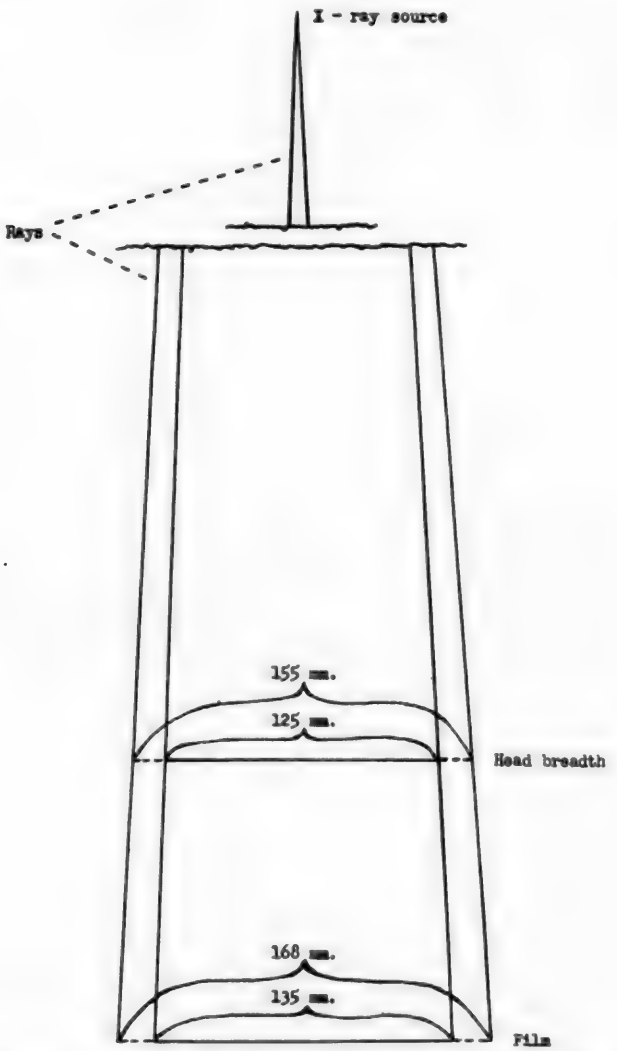

Fig. 2.—Schematic diagram illustrating differential distortion resulting from x-ray divergence at head-breadth-to-film distance of approximately 115 millimeters. Note that for the two head breadths of four-year-old children shown, magnification is 10 mm. on the smaller and 13 mm. on the larger.

2. The records from the direct measurement procedure were more valid than those from the radiographic procedure.

FINDINGS FOR BIGONIAL DIAMETER

Reliability of Measurements.—The data for bigonial diameter were described earlier. Succinctly, they consisted of four series of records, two each from roentgenographic and direct measurement, amassed independently on the same sample of ninety-four children between 4 and 5 years of age. The analyses to which they were subjected paralleled those for biparietal diameter. That is, reliability of bigonial diameter as determined by each of two methods was studied in terms of (a) reliability coefficients and (b) distributions of differences.

The reliability coefficient obtained from correlation of the roentgenographic measurements of J. W. P. and R. D. D. was $r = .989$. A definitely lower degree of relationship was found from the direct measurements of H. B. and H. V. M. ($r = .926$).*

The differences between the records of each pair of anthropometrists (disregarding sign) were distributed as follows:

DIFFERENCE INTERVAL	X-RAY METHOD		DIRECT METHOD	
(MM.)	N	%	N	%
0 to 0.9	83	88	27	29
1 to 1.9	11	12	34	36
2 to 2.9			25	27
3 to 3.9			7	7
4 to 4.9			1	1

For each of the ninety-four roentgenograms measured, the difference between the records of J. W. P. and R. D. D. was less than 1.8 millimeters. In contrast, over 40 per cent of the separate measurements of H. B. and H. V. M. differed by 1.8 mm. or more.

As for biparietal diameter, two independent roentgenograms taken on each of six children were used to estimate the unreliability involved in placing the subject in the head positioner and sliding the cassette into position. With the mean from fifteen bigonial measurements on a roentgenogram being used as the "true" width of its shadow, the difference between each pair of exposures was less than 0.3 millimeter. The average difference approximated 0.1 millimeter.

Summarizing, records of bigonial diameter on children of preschool age are less reliable by the method of direct anthropometry than by the method of measuring posteroanterior roentgenograms. Employing the latter method, two technicians working independently can obtain records which differ by no more than 1 mm. for more than seventy subjects in one hundred, and by no more than 2 mm. for practically all subjects.

Comparability and Validity of Methods.—The same steps being followed as with biparietal diameter, two series of values were obtained to characterize the bigonial diameters of the subjects by each method. The succeeding tabulation

*Applying the appropriate test of statistical significance: the coefficients differ by .063, the standard error of difference approximates .015, and the significance ratio is 4.2.

presents the findings from analysis of these values for central tendency and variability.

METHOD	N	MEAN	STANDARD ERROR OF MEAN	STANDARD DEVIATION	STANDARD ERROR OF SIGMA
Direct	94	85.6	±0.4	4.2	±0.3
X-ray	94	79.9	±0.4	4.2	±0.3

It will be seen that:

1. Direct measurements of bigonial diameter tend to be larger than roentgenographic measurements. The mean of the values for the x-ray method is smaller than that for the direct method by 5.7 millimeters.

2. Variance is no greater from one series of values than from the other. The standard deviations are 4.2 mm. in both instances.

These findings indicate that: (a) the thickness of soft tissue included in direct measurement exceeds the amount of radiographic magnification, and (b) the factor of differential distortion in the roentgenographic method registers no greater influence on variability than the factor of unreliability in the direct method.

Magnification was studied on the child's skull illustrated in Fig. 1. The mean of six independent records taken by three anthropometrists gave a bigonial diameter from direct measurement of 66.0 millimeters. A metal pin was attached to each landmark, the skull oriented in the head positioner, the pin to cassette distance measured (78 mm.), and a posteroanterior roentgenogram secured. Breadth of the bigonial shadow, as determined by three anthropometrists independently, was 68.7 millimeters. The amount of magnification (2.7 mm.) is approximately one-fourth that obtained on the same roentgenogram for biparietal diameter. It need not be labored that, inherently associated with this lesser magnification, there is markedly reduced differential distortion.

SUMMARY AND RECOMMENDATIONS

Study is made of the technical and morphologic adequacy of data collected by two procedures. One procedure calls for direct measurement of the child; the other involves radiography and measurement of the roentgenogram. Both procedures are carried through on a high plane of scientific rigor. The data are for two dimensions of the calvaria and face, biparietal diameter and bigonial diameter. Mainly, the sample consists of ninety-four white children between the ages of 4 and 5 years. Supplementary use is made of a child's skull, estimated age 4 years.

Selected findings are: (1) biparietal diameter is measured with high reliability by both procedures; (2) bigonial diameter is measured more reliably by the roentgenographic procedure than by direct measurement; and (3) biparietal diameter is depicted more validly by direct measurement than by the roentgenographic procedure.

Consideration of the full reliability and validity findings for each dimension warrants two recommendations. It is recommended that in growth studies on the young child (1) biparietal diameter be taken by direct measurement, and (2) bigonial diameter be taken from measurement of the posteroanterior roentgenogram.

HISTOPATHOLOGY OF THE DENTAL PULP OF DOGS FOLLOWING EXPOSURE

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THIS study was undertaken in order to ascertain more fully the response of the dental pulp which has been subjected to injury. A number of reports dealing with this topic in its various phases have already appeared. These investigations have been concerned with the effects of traumatic stimuli,[1] response to various filling materials,[2-4] and also the reaction of certain drugs.[5, 6] Several investigations concerned primarily with methods of clinical treatment[7-9] of the pulp have also been carried out.

Studies also reported by Chase,[10] Salter,[11] Caush,[12] Davis,[13] and Grove[14] have added considerably to the sum of knowledge concerning this phenomenon and, in addition, have been an impetus to explore further the complexities of pulp physiology and pathology.

Some time later, Feldman[15] and Neuwirt,[16] in a series of experiments concerned with the biologic method of pulp treatment, reported closure of the exposed root canal by vital hard tissue (apparently dentin) following the covering of the pulp with dentin powder. Similar findings have been reported by Gottlieb,[17] Hellner,[18] Muller,[19] and Rzestarski.[20]

MATERIALS AND METHOD

Our experimental material consisted of three dogs. In all operative procedures, asepsis by means of the rubber dam and instrument and operative field sterilization was attempted although not always successfully maintained. Cavity preparations of approximately 3 mm. diameter and of uniform depth were made into the coronal dentin of seventeen teeth. A geared-down handpiece was used so as to minimize traumatic stimuli. Three teeth were then sealed with gutta-percha and reserved as control material. In all the remaining teeth a small, round burr was directed into the cavity base and the pulp exposed. It was not possible to ascertain the amount, if any, of dentin chips or filings forced into the pulp by the burring procedure. As soon as the slightest "give" of the instrument was felt, signifying entrance into the pulp chamber, the burr was carefully removed. The resulting hemorrhage was wiped away with cotton pellets and a small, round disk of tin foil placed on the cavity floor so as to cover the exposure channel. A seal of gutta-percha or oxyphosphate of zinc cement completed the operation.

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The time intervals from operation to animal sacrifice ranged from seven to forty-seven days. Upon the termination of the experiment, intraoral radiographs were taken of all the teeth by means of Koch's technic.[21] The teeth were then removed from the jaws and immediately ground by stone until the apical pulps were nicked. Fixation in 10 per cent formaldehyde was thereby accelerated. Following decalcification and celloidin embedding, longitudinal sections were cut serially, mounted, and stained with hematoxylin and eosin.

In an attempt to use the guinea pig as an additional experimental animal, a burr was directed into the inferior border of the right or left mandible so as to penetrate the basal third of the incisor tooth. The difficulty of properly guiding the instrument to accomplish this end is attested by the fact that of six attempts only three were successful. Use of this animal was, therefore, discontinued.

OBSERVATIONS

The pulpal tissues of the fourteen exposed teeth show striking changes which assume particular significance when reviewed in order of their appearance from seven to forty-seven days.

The lesions produced in a lower left third premolar by an exposure of seven days' duration (Fig. 1) presents the following histological picture: The operative channel, loosely filled with necrotic material, is in direct communication with a large coronal abscess. A small spicule of dentin, depressed in a pulpal direction, retains its attachment with the pulpal margin of the dentinal wound. Odontoblasts are intact for a brief distance to the right and left of the site of exposure, although smaller than usual, and contain pyknotic nuclei. They are replaced throughout the remainder of the coronal pulp by a bandlike arrangement of fibrous connective tissue. Odontoblasts in the radicular pulp are of normal appearance. Peripheral to the abscess area, particularly in a coronal direction, the pulpal tissues are markedly hyperemic, and there appears to be a formation of new capillaries and fibroblasts. Also present are several masses of osteoid-like tissue composed of an amorphous, granular, purple-staining material arranged in islet fashion (Fig. 2). An occasional fibroblast, as well as several capillary sprouts, can be found in the substance of the amorphous structure. A further finding is an isolated concretion of typical dentin with clearly demonstrable tubular architecture (Fig. 3). Arrayed along the basal surface of this formation are oval- and stellate-shaped cells, some of which send fiberlike extensions into the tubules. Several other cells have the appearance of typical fibroblasts.

The coronal pulp tissue of a lower right third premolar (Fig. 4), similarly exposed for seven days, shows, in addition to acute abscess formation, a dense packing of dentin spicules and osteoid-like formations similar to those previously described. These bodies are bound together in compact fashion by an extensive network of interlacing fibroblasts and collagenous fibers accompanied by a rich vascular supply. A great number of small and large round cells are distributed throughout the section. No odontoblastic layer is present in this field. In its stead is a layer of fibrous tissue containing many engorged capillaries and ex-

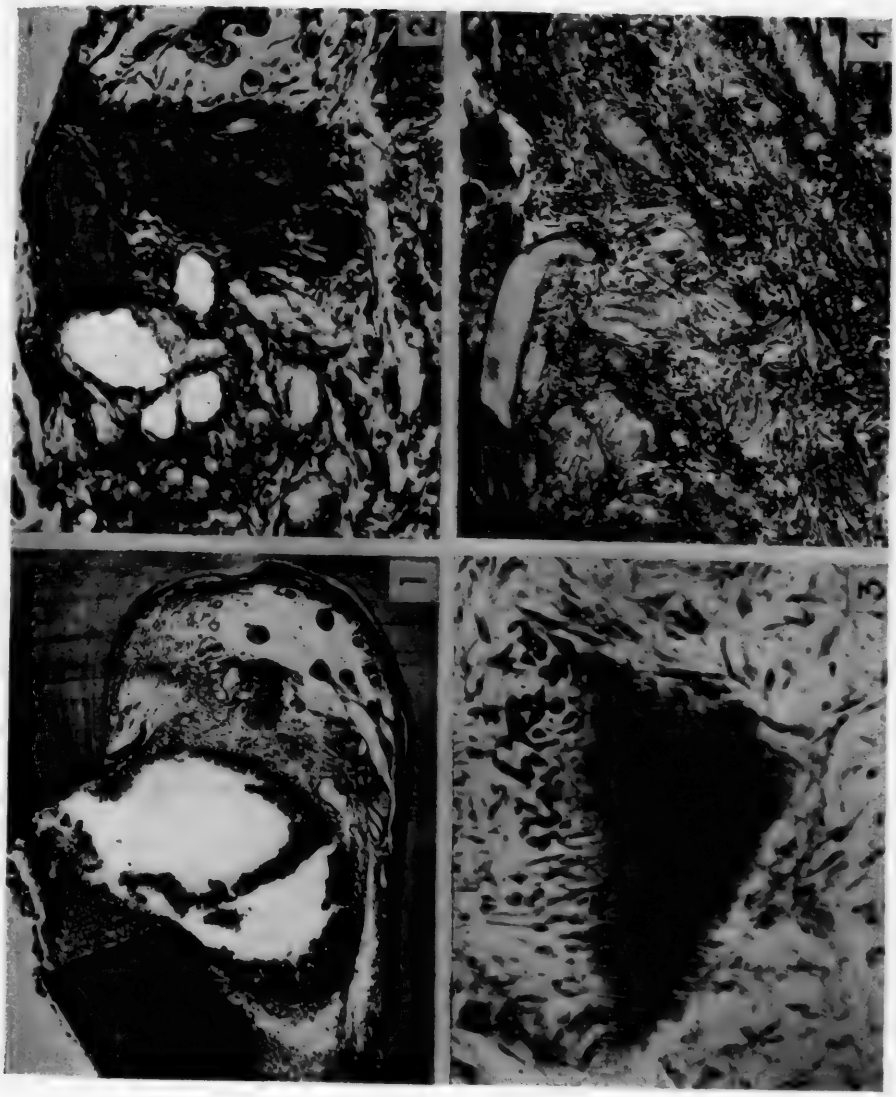

Fig. 1.—Lower left third premolar exposed for seven days showing large coronal abscess.
Fig. 2.—High-power magnification of osteoid-like tissue in pulp of same tooth.
Fig. 3.—High-power magnification showing isolated formation of typical dentin.
Fig. 4.—Coronal pulp tissue of a lower right third premolar, similarly exposed for seven days, showing numerous dentin spicules and osteoid-like formations.

travasated red blood cells. As the radicular pulp (not shown in this photomicrograph) is approached, the tissue assumes a more normal appearance. The odontoblasts first become evident as a flattened layer of cells containing karyolytic and pyknotic nuclei and the vascularity is moderately excessive. The connective tissue elements are mucoid in character and cellular infiltration is nil.

A later change, as denoted in the pulpal tissues of a lower right, fourth premolar with an exposure of fourteen days' duration, is shown in the next series of figures. At both pulpal margins of the exposure channel are shown large dentin splinters that project into the underlying pulp tissue retaining their organic union with the intact primary dentin (Fig. 5). These "greenstick" fractures apparently occurred when the revolving burr had almost reached the pulp and were the result of the slight pressure required to break through the remaining thin wall of dentin. Such a finding is consistent in all teeth of this experimental series.

Beneath the exposure channel, the coronal pulp shows massive round cell infiltration with correspondingly numerous fibroblasts, endothelial cells, and capillary vessels. Also present, but better demonstrated in later photomicrographs, are a number of clearly defined osteoid-like formations bounded, in capsular fashion, by fibroblasts and containing in their midst a number of irregularly shaped islands of tissue closely resembling dentin. These are, for the greater part, amorphous and granular in appearance but show a faint, unmistakable tubular architecture. The matrices of these osteoid structures are composed of a homogeneous, pink-staining material containing occasional lacunae.

On the pulpal wall, opposite the site of exposure, is an area of pathosis (Fig. 6) which, by evidence of position, might conceivably have been caused by the operating burr. The lesion is characterized by a saucer-shaped formation of irregular secondary dentin demarcated from the primary by a prominent incremental band. The pulp tissue is markedly hyperemic, and numerous round cells and extravasated erythrocytes are scattered throughout. Fibroblasts are, likewise, present in excessive numbers, and the odontoblastic layer shows such degenerative changes as vacuolization, crowding of adjacent cells, and karyolysis of nuclei.

Photomicrographs of an upper left fourth premolar, exposed for twenty-six days, are seen in Figs. 7 through 10. The first of these is a longitudinal section through the plane of the exposure channel. Projecting into the pulp from the right inferior margin of the perforation is a large spicule of dentin similar to that observed in the lower right fourth premolar. A much smaller spicule, bent backward in a horizontal position, is seen at the left inferior margin of the orifice. Both these dentinal fragments retain organic union with the primary dentin. Underlying the larger splinter, and demarcated from it by a distinct incremental line, is a triangular formation of regular secondary dentin. The subjacent odontoblasts are intact and of normal appearance, whereas elsewhere in the coronal pulp they are, for the greater part, replaced by fibrous tissue. The pulp in the immediate vicinity of the exposure shows evidence of early granulation tissue formation. Apically, to the left of the section, is a large oval area devoid of any cellular elements but bordered by a concentration of necrotic material and poly-

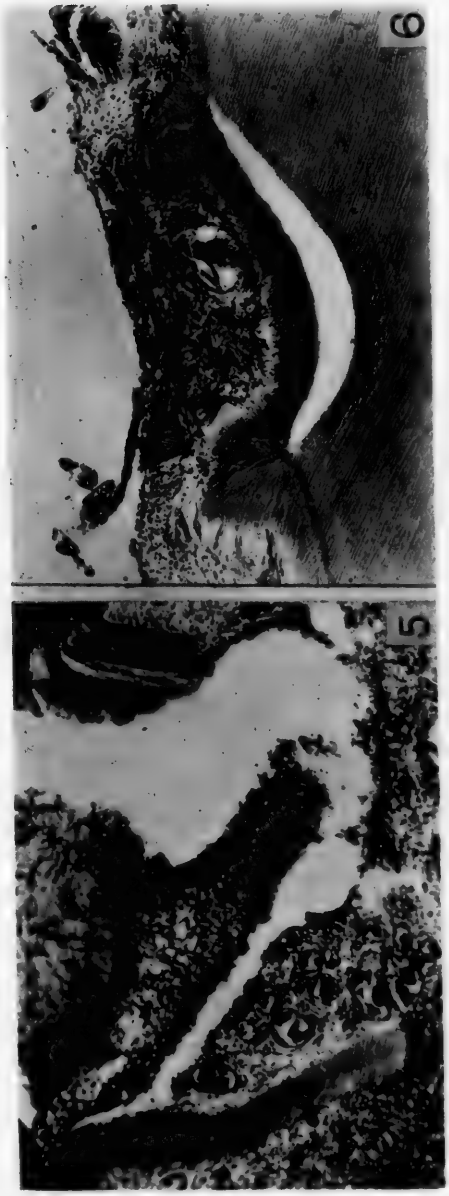

Fig. 5.—Lower right fourth premolar showing exposure channel into the pulp. Exposure duration of fourteen days.
Fig. 6.—Same tooth as seen in Fig. 5 showing area of pathosis on pulpal wall opposite site of exposure.

morphonuclear and round cells. This large abscess formation extends as far as the dentinal wall where it is limited by the fibrous band which has replaced the odontoblastic layer. Occupying a centric position in the coronal pulp is a large, compact mass of osteoid-like tissue (Figs. 7 and 8), the architecture of which is best seen by study of a small segment under high-power magnification (Fig. 9). Forming the wall of the structure is a thick layer of tissue resembling irregular dentin and bordered on its external surface by a number of stellate-shaped cells. The core of the mass is honeycombed in appearance, a result of the entrapment of numerous spicules of dentin that apparently have undergone varying degrees of degeneration or dissolution.

As in the case of the lower right fourth premolar, the pulpal tissue and dentinal wall opposite the exposure wound show changes that could have been brought on, inadvertently, by the burr tip encroaching upon these tissues. A high-power magnification (Fig. 10) shows the hyperemic state of the pulp and a marked round-cell infiltration. A round concretion of dentin lies free in the area. Projecting in polyp fashion from the subjacent dentin surface is a similar formation bordered on its periphery by a single layer of cells resembling odontoblasts. The circumpulpal layer of dentin, which gives rise to this outgrowth, is irregular in architecture and separated from the normal primary dentin by a prominent incremental band partially split by artifact in this region. No evidence of odontoblasts is seen to right or left of the polyp formation. Rather, there is replacement by fibroblasts and connective tissue fibers.

Formation of a large osteoid-like mass that almost occludes the pulp canal is observed in an upper left third incisor exposed for thirty-six days (Fig. 11). When viewed under higher magnification (Fig. 12), many scattered islands of irregular size and shape are seen to be bound together by a bright pink cementing matrix of more or less homogeneous appearance. Detailed examination of the latter tissue discloses an irregular, sparse arrangement of dentinal tubules in which an occasional cytoplasmic fiber may be found. It is possible to follow the course of several such filaments to their cell bodies arranged along the periphery of the dentin formation. However, the odontoblast-like cells do not constitute a uniform layer but rather are arranged in short, isolated groups, leaving much of the dentin surface bare of these elements. Whereas the cementing portion of the structure being described is typical of irregular secondary dentin, the entrapped islands of purple-staining tissue have the appearance of normal primary dentin. This finding, in addition to the spicular outline of these bodies, suggests the possibility that they were formerly a part of the coronal dentin proper and had been introduced mechanically into the pulp by the operative burr.

Reverting to the low-power photomicrograph of this tooth (Fig. 11), a large abscess area is seen below the exposure channel. The sparsity of material in the core of the lesion is undoubtedly an artifact attributable to the technical procedure in preparing the section. Nevertheless, some necrotic debris and numerous pus cells are still visible. A distinct wall of fibrous tissue limits the extent of the abscess and the surrounding pulp tissue is markedly infiltrated with small and large round cells. In addition, there is a greatly increased vascularity and

Fig. 7.—Longitudinal section of an upper left fourth premolar exposed for twenty-six days showing exposure channel, abscess formation, large calcified concretion, and injury to pulpal wall opposite exposure wound.

Fig. 8.—High-power magnification of the pulpal mass seen in Fig. 7.

Fig. 9.—Demonstration of detailed architecture of mass in Fig. 8.

Fig. 10.—High-power magnification of dentinal wall lesion opposite exposure channel.

extensive extravasation of erythrocytes. Numerous fibroblasts are also in evidence, as well as several small spicules of dentin. An advanced stage of fibrosis with countless round cells enmeshed between the fibers is present in the zone directly underlying the inferior border of the abscess. A corresponding pulpal condition exists on the apical side of the large dentin mass already described. Despite the fact that no distinct cell layer resembling odontoblasts is seen in this region, there is a wide band of newly formed secondary dentin. The pulp tissue in the apical third of the tooth is free of fibrotic change but shows a hyaline type of degeneration and vacuolization of the odontoblasts.

Among the interesting changes seen in an upper left canine exposed for forty-seven days is one which vividly demonstrates the consistent finding, mentioned previously, of irregular secondary dentin formation beneath the depressed fracture segments at the right and left inferior borders of the exposure channel (Fig. 13). The orifice proper is capped by a quantity of necrotic material and the underlying pulp shows new granulation tissue formation, massive round-cell infiltration, and large areas of hemorrhage. A large spicule of dentin is also seen in the lower right corner of the field. As the midradicular region is approached, a distinct zone of transition occurs in the pulp picture from extensive round-cell infiltration and phagocytic macrophage activity on the left, to normal pulp tissue on the right (Fig. 14).

It was stated in the introductory comments to this report that the guinea pig was used in several of the experimental procedures, but that it was discontinued because of certain technical difficulties. Nevertheless, the few interesting findings that were noted are presented at this point.

Fig. 15 shows a section of a lower left incisor that had been injured by a burr introduced via the mandible and thence through the basal third of the tooth. The time interval from operation to death of the animal was thirty-eight days. It is of interest to note the typical fracture effect caused by the burr. It bears a striking resemblance to the experimental fracture picture in the rat reported by Sarnat and Schour.[22] For purposes of orientation, the following structures are identified. The proximal root segment is to the left with invaginating periodontal tissue separating it from the distal tooth segment on the right. Several wide, irregular increments of secondary dentin seal the apical section of the pulp. Incorporated within the whirllike formation of dentin are several islands of amorphous, deep purple-staining material. On the other hand, the pulp tissue of the distal segment is in direct communication with the site of fracture. There is, however, a beginning infiltration of the pulp by fibrous connective tissue from the fracture zone. Aside from a moderate degree of fibrotic change and some hemorrhage, the pulp is not particularly unusual.

Examination of the epithelial elements shows additional striking changes. The enamel, which is normally found on the outer, convex surface of the incisor, is completely absent in the region of the operative wound. It is present, however, on the intact root surfaces, although not particularly evident on the proximal segment where its former position is denoted by an artifact of decalcification. A high-power magnification (Fig. 16) demonstrates the severe disturbance

Fig. 11.—Upper left third incisor exposed for thirty-six days showing abscess formation and osteoid-like mass in pulp.
Fig. 12.—High-power magnification of large pulpal concretion.

Fig. 13.—Upper left canine exposed for forty-seven days showing exposure wound.
Fig. 14.—High-power magnification of pulp of tooth in Fig. 13 showing cellular reaction.

to ameloblasts in this area. Aside from their striking morphological change, characterized by pyknosis and karyolysis, there is a peculiar arrangement of the cells in semilunar groups, each overlying a mass of globular, enamel-like material. Farther apically, at some distance from the site of injury, is an area of complete arrest of enamel formation (Fig. 17). Mounds of enamel matrix are seen on either side of the defect and the ameloblastic layer is completely separated from the proximal enamel surface. A pale-staining strip of intact cells remains over the enamel to the right of the hypoplastic zone. No explanation is offered for the peculiar arrangement of the raised cell layer which does not appear to be an artifact.

Radiographs of the dog teeth failed to disclose any evidence of pulp calcifications or periapical pathosis.

DISCUSSION

To interpret the findings herein reported would be to conjecture on cause and effect which is not the purpose of this paper. That pulp tissue follows the general biological principles of healing and repair is, however, undoubtedly true, as demonstrated by past studies. In this connection, however, the various factors relative to anatomical structure such as diameter of apical foramina, size of pulp canal, degree of blood supply, and so forth, all of which bear a relationship to physiological and pathological mechanisms, must be taken into consideration. Thus, to formulate completely the history of pulp response to injury, exhaustive studies incorporating all phases of biology are essential. The scope of the present report is, therefore, to present merely a histological sequence of events in dog teeth following a particular type of injury. It is apparent that the changes described follow the generally accepted concept of injury and tissue response.

The earlier or acute changes, as seen in the seven-day exposure, exhibit necrosis, abscess formation, and vascular response with exudation. Stages of repair by organization are seen in the longer exposures where there is a typical picture of granulation tissue formation proceeding to fibrosis. It is apparent that with the degree of injury sustained by the pulpal tissue, the mechanism of repair cannot be one of resolution with restitution to the original normal architecture. Such repair would of necessity follow a less severe injury involving a single cell type or element, or a simple parenchymatous change with the cell in a reversible stage retaining the ability to return to normal structure and function.

Confirming observations of other investigators,[15, 16, 24] and apparently playing an important part in the repair mechanism of exposed pulps, is the presence of dentin fragments in the pulp tissue which appear to function as nidi for the formation of additional calcified tissues resembling dentin. The question is then raised as to the role played by artificially introduced dentin fragments in accelerating such a process in exposed pulps and the method by which such action is accomplished. Since other investigators have described similar pulp changes following introduction of such substances as ivory powder and cement,[17] it would appear that the mechanism of calcification is typical of pulpal tissue response

Figs. 15, 16, and 17.—Pulp, dentin, and enamel changes in injured guinea pig incisor.

to a given foreign body. However, certain of the photomicrographs in the present report indicate an additional possibility, namely, that viable dentin introduced into the pulp at the time of exposure may also simulate the action of a transplant.

SUMMARY

A histological report describing pulp changes observed in fourteen dog teeth following exposure intervals of seven through forty-seven days is reported. The pulp tissues show gradations of inflammatory response ranging from acute to chronic which are characterized by necrosis, abscess formation, vascular response with exudation, and granulation tissue formation proceeding to complete fibrosis. The most striking change was the presence of osteo-dentinlike formations surrounding spicules of dentin introduced into the pulp during the operative procedures. The question which is raised relative to the mechanism of this repair process is whether it is indicative of a foreign body tissue response, or whether it is due to the transplant of viable tissue. This latter statement needs further investigation for clarification.

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SALIVARY INHIBITION AND DENTAL CARIES

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IT WAS reported by Bloomfield,[10, 11] who had exhaustively studied the problem of natural immunity, that foreign bacteria artificially introduced into the oral cavity were rapidly eliminated. He expressed the opinion that, except for the normal flora, bacteria do not as a rule grow freely on the mucous membranes of the mouth and throat, and that special conditions, either local infection or transient invasion, are necessary to account for the presence of foreign organisms.

Bloomfield also reported that the normal flora is not eliminated but continues to flourish in the buccal secretions following washing or copious irrigation which rapidly eliminates adventitious organisms. He concluded that any organism which persists on the normal mucous membrane for more than a few hours must have a biologic relationship to the mucous surface. Otherwise, it would be removed as a foreign particle by the normal eliminative mechanism. Bloomfield, while undoubtedly dealing with an antibiotic mechanism, failed to recognize the significance of his observations, and considered that foreign bacteria were eliminated mechanically.

The work of Dold[14] and of Weigmann and Koehn[14] cast additional light on the normal oral bacterial and bacteriostatic mechanism and also on its method of operation. These investigators showed that normal human saliva contains factors that not only prevent the multiplication of certain pathogenic organisms, but often will also kill them in a few hours. They demonstrated that virulent diphtheria organisms, not killed by contact with these normal salivary constituents, are almost invariably transformed into avirulent types. They erroneously presumed that the inhibitory agent was a lysozyme. Weigmann and Koehn[32] found that these transformed "mutants" will breed as apparently pseudodiphtheric organisms for at least ten transplants in an ordinary medium.

The presence of the "inhibins" or "bacteria-transforming enzymes" in normal saliva has been confirmed by a study of salivary inhibition and transformation of numerous other bacterial species. Wehrse[31] for example, found that, in the presence of normal human saliva, hemolytic streptococci are often killed in from five to six hours and, if not killed, are almost invariably transformed into the nonhemolytic variants. Pesch and Damm[24] report a similar bactericidal and transforming action on Type I and Type II pneumococci. The inhibins in normal saliva are more or less completely removed by Seitz filtration, are nondiffusible through animal membrane, and are destroyed quantitatively by the heating of saliva to 56 degrees C. and by its being maintained there for thirty minutes. Saliva obtained directly from the salivary glands does not have this inhibitory property.

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Muhlenbach[21] has demonstrated that this salivary inhibition is due chiefly, if not entirely, to the growth of the normal salivary streptococci in the saliva. Washings of a blood-agar culture of salivary streptococci produce an inhibition identical with that derived from whole saliva. In contrast to the streptococci, the other members of the basal oral flora cause no such inhibition. Hill[18] demonstrated that the inhibitory property of the saliva obtained from individuals who were immune to dental caries was considerably greater than that occurring in saliva obtained from individuals in whom dental caries was rampant.

Recently, Thompson and Shibuya[28] have not only substantiated Muhlenbach's findings on salivary inhibitions, but they have also conclusively demonstrated that the inhibitory property of saliva is derived almost exclusively from the nonmucoid-producing streptococci occurring in the saliva.

Belding and Belding,[4] and Belding[2] have shown that the presence, in large numbers, of the mucoid-producing streptococci in the saliva is indicative of susceptibility to dental caries; and that by dietary methods (essentially the deletion of sucrose), dental caries can be arrested in from three to six weeks with a concomitant disappearance of the mucoid streptococci and the reappearance of the normal salivary streptococci which impart to saliva its significant inhibitory properties. This evidence at least partially explains why many of the infectious diseases such as scarlet fever and diphtheria, which are constantly endemic in civilized communities exhibit with dental caries an apparently capricious geographic distribution, being common in the temperate zone and rare or unknown in the arctic regions and the tropics.

In this connection, Kleine and Kroo[19] have reported that diphtheria has not been known to occur in the interior of Africa and that scarlet fever is extremely rare. The *Journal of the American Medical Association* has reported editorially:[15]

> "For some thus far unexplained reason, scarlet fever is comparatively rare in the tropics. The disease has been repeatedly introduced into India, South America and the Philippines. Invariably only a local epidemic resulted, largely confined to newly arrived foreigners, and with a few cases developing among the native population."

Heinbecker and Irvine-Jones[17] also reported that scarlet fever and diphtheria are unknown diseases among the primitive Eskimos. Asbelew and Margo[1] reported that diphtheria and scarlet fever have never been known to occur on the Arctic Island of Kolgujew. Milam and Smillie[20] and Burky and Smillie,[12] as a result of their investigations, stated that the absence of *Streptococcus pyogenes* is apparently a characteristic of isolated populations. Further, the infections commonly associated with this organism, such as scarlet fever, erysipelas, tonsillitis and puerperal sepsis, were rare in the areas covered by their studies. It is also recognized that the incidence of dental caries is low in the tropics and almost negligible among the isolated Eskimos, yet Waugh[30] reports that shortly after the introduction of sugar into the diet rampant dental caries makes its appearance.

Before the carious lesion occurs, an alteration in the oral flora must have taken place. The nature of this change can be at least partially explained by

the fact that sucrose forces mutations in some of the streptococcal cells of the basal flora. In this connection, Pryer[25] reported that he was able to produce mutation in streptococci by growing them in the presence of this sugar. Oerskov[22] (1930) and Oerskov and Poulsen[23] (1931) discovered that certain streptococci formed mucoid colonies when grown on agar containing sucrose. In 1938, we reported that *S. odontolyticus,* which is always associated with caries acuta, forms a mucoid colony on sucrose agar.[4] Niven, Smillie, and Sherman[26] investigated this phenomenon and reported that, with the exception of raffinose, sucrose was the only sugar on which the streptococci, under study, formed mucoid colonies. More recently, Sugg, Hehre, and Neill[27] have demonstrated that the antigenic structure of certain streptococci was altered by their being grown sucrose broth cultures. These changes did not occur when the same organisms were grown in glucose broth. Sucrose caused these organisms to develop characteristic polysaccharides.

This change in the pathogenicity of the oral streptococci resulting in their subsequent ability to cause disease is not unique, for similar changes have been observed to take place in the majority of the other bacteria causing human disease. These changes are recognized to be of the utmost significance in explaining the epidemiology and natural history of many microbic diseases.

The following dietary regime, which tends to revert the activated mucus-producing oral streptococcus to the primitive level, will usually inactivate the caries-producing factors in the most stubborn cases of dental caries.

1. A twenty-four-hour fast period; water, plain tea, and coffee may be taken in any amounts.

2. A period of forty-eight hours during which two to three quarts of cultured milk is consumed daily. This is followed by a week in which an essentially protein diet is administered and may consist of the following items: beef, mutton, pork, poultry, fish, eggs seafood, shellfish, butter, and cream. The consumption of cultured milk should be continued. All sugar-cured meats must be avoided.

3. After ten days, vegetables and fruits should be included in the diet and consumed in quantities to suit the appetite. However, those foods containing 12, 15, 16, and 20 per cent carbohydrate should not be included for the first three weeks. If the patient demands a sweetening agent, saccharine should be supplied. Usually after following this therapeutic diet for four weeks, the patient can be returned to a normal nutritionally adequate diet and remain immune to dental caries until the bacterial balance is again disrupted.

No foods but those mentioned should be included in the diet, for it has been repeatedly demonstrated that the use of such insignificant things as cough drops, mints, chewing gum, or soft drinks may defeat the purpose of the diet. Figs. 1 and 2 show the bacterial picture before and after treatment.

In that autarcetic immunity[16] has far greater implications than the presence or absence of dental caries, the diet used in the arrest of dental caries may have far-reaching medical implications.

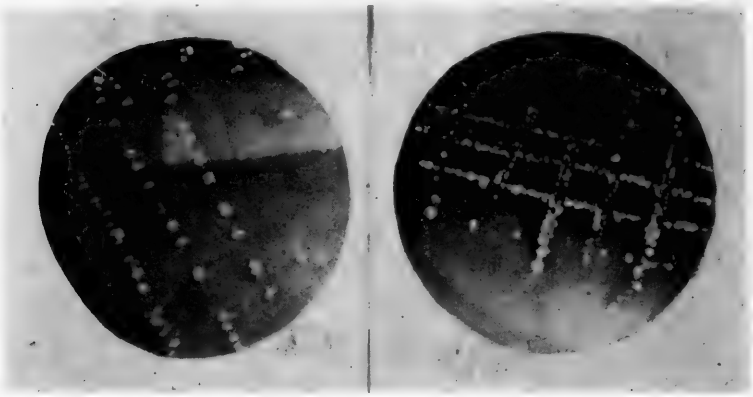

Fig. 1.—Demonstrating the colonial formations procured by streaking saliva of an individual affected with caries acuta on nutrient blood agar containing 2 per cent sucrose. Note hemispherical mucoid colonies.

Fig. 2.—Same case as Fig. 1, showing colonial formation (flat) at conclusion of treatment. Note absence of mucoid colonies.

DISCUSSION

Under natural conditions, there has evolved in the alimentary tract a basal flora which is in perfect harmony with its environment. It is vegetative and lives in close biologic relationship to the mucous membranes of the host. Bacteria respond to a perfect nutritional, chemical, and biochemical environment by developing in the direction of increased stability, increased resistance, progressively lessened pathogenicity, and, eventually, absolute vegetation.

It is apparent at the present time that the majority of the bacteria constituting the basal flora of civilized man are in a constant state of flux, which is expressed both functionally and morphologically. Whatever state a given organism is in at the moment is largely dependent on its immediate previous environment. While there are certain forces which drive bacteria into involution and a vegetative existence, there are other forces which drive them in the direction of evolution and increased pathogenicity.[7]

In commenting upon the significance of bacterial evolution, Topley and Wilson[29] stated:

> "Taking the experimental evidence as a whole, it would seem to accord with the view, expressed by many epidemiologists, that variations in the character of the parasites are of major importance in the spread of human infections, and that an outbreak of disease may be initiated by the evolution, or importation of an 'epidemic strain' of the causative organism."

SUMMARY

1 Under natural conditions, the body has a mechanism which eliminates transforms, or kills many types of pathogenic organisms.

2. Within recent years, civilized human beings have encountered an environmental factor which makes caries acuta an almost universal disease and concomitantly reduces the effectiveness of the bactericidal and bacteriostatic mechanism of the saliva.

3. Sucrose, because of its inhibitory action on bacteria and the resultant mutations, is probably the most important environmental factor which has interfered with functioning of the oral autarcetic immunity mechanism.

4. It is suggested that the high incidence of scarlet fever, diphtheria, dental caries, appendicitis, and other diseases of civilized man might be partially explained by the difference, bacteriologically, which exists between modern and primitive communities.

5. A diet is given which eliminates from the basal flora the mucus-producing streptococci, arrests dental caries, and, by stimulating the inhibitory principles of Muhlenbach, tends to enhance the autarcetic immunity mechanism.

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SEX AND DENTAL CARIES IN ALBINO RATS (RATTUS NORVEGICUS)*

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THE writers and their collaborators have been investigating since 1931, the roles of various factors in producing dental caries in the lower molar teeth of albino rats. The physical properties of the food,[1-3] age,[3] and heredity[4-9] have been proved to participate in the development of dental caries in rats. Acid-forming bacteria and fermentable carbohydrates are believed to be very important. Does sex in rats contribute to resistance to tooth decay?[1]

A caries-resistant strain and a caries-susceptible strain have been created by phenotypic selection, progeny testing of breeding pairs, and brother x sister inbreeding.[4-9] As soon as the rats became 35 days of age they were given a caries-producing diet[1, 2] and were examined fortnightly for cavities in the molars of the lower jaw. The measure of an animal's resistance was the caries time—the number of days it consumed the cariogenous diet until the first cavity appeared in a lower molar.

The susceptible line was not used for this study because variation within it was so low that even if it were true that males and females actually differed, on the average, from one another, the necessarily small difference might not be

TABLE I

CARIES IN RESISTANT MALE AND FEMALE RATS*

	MALES		FEMALES	
GENERATION	NUMBER	AVERAGE CARIES TIME (DAYS)	NUMBER	AVERAGE CARIES TIME (DAYS)
6	83	243	89	219
7	123	234	130	247
8	130	181	132	203
9	73	313	91	333
10	159	276	169	290
11	140	372	133	377
12†	145	283	144	319
	Total 853	Average 272.3±3.8 days	Total 888	Average 285.6±3.6 days

*Animals dying before they developed caries are omitted.

†Rats still living that had not developed caries were omitted.

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*Dr. William J. Gies suggested to the writers that the extensive data collected in the study of the inheritance factor affecting dental caries in rats be used, also, to discover whether sex influences resistance to caries.

statistically significant. Consequently, the sixth to the twelfth generations, inclusive, of the resistant animals were used. By the sixth generation the differentiation between susceptible and resistant rats had been established, and the twelfth generation of resistant rats is the latest one in which almost all the records of individual animals have been computed.

There were 853 male and 888 female rats. The average caries time for the males was 272.3± 3.8 days, and for the females, 285.6± 3.6 days. The difference between these means was 13.3± 5.2 days. Since this difference was only 2.6 times as large as its probable error, it was not statistically significant. In the sixth generation, the average for the males exceeded that for the females, though the reverse was the case for all the remaining six generations.

One might suspect that, though the difference between the males and females was not statistically significant in a conventional sense, yet the consistently higher resistance of the females indicated a slight contribution of femaleness toward the prevention of dental caries. Males and females were fed and handled alike, so that source of difference was eliminated. A minority of females bore young, but it seems probable that reproduction would decrease resistance to caries rather than increase it.

Might not a differential death rate account for the difference between the sexes? There are thirty-five more females than males in the table. Could it be that sickly infant and juvenile males which died early in life would have been, had they lived, more resistant to caries than the more vigorous surviving males and females? There is no evidence, as far as we know, that low physical vigor is positively correlated with resistance to dental caries. It might be suggested that the mortality rates at various ages for our recorded males were higher than for the recorded females, so that the proportion of males which survived long enough to develop caries was less than for the females. Rats that died before developing caries were not included in the table. The average age at death of the 130 males that died before showing cavities in the molars was 478 days, while the 160 females that died before caries appeared died at an average age of 438 days. These males lived longer than the females, and so had the greater opportunity for the acquisition of cavities.

The difference between the means for the sexes was only 13.3 days. The teeth were examined, as a rule, every two weeks, so that the difference is equivalent merely to one unit of time. Scrutiny of the data leaves room for suspicion that sex may sometimes be a minor factor in resistance to dental caries in albino Norway rats. If femaleness increases resistance to caries, its influence is relatively slight and the accurate measurement of that influence would require more refinement of technic.

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MICROORGANISMS ASSOCIATED WITH DENTAL CARIES IN THE COTTON RAT*

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INTRODUCTION

PREVIOUS studies from this laboratory have shown that a high incidence and extent of carious lesions in the molars of cotton rats occurs when these animals are fed purified rations high in sucrose or other fermentable sugars.[1,2] On the other hand, a low incidence of caries occurs in animals fed a stock ration, or experimental diets containing dextrin in place of sucrose.[2] Since the occurrence of certain oral bacteria has been associated with dental caries and a correlation has been found to exist between the number of lactobacilli present and caries susceptibility in human teeth,[3-5] it seemed important to determine whether or not such a correlation existed between the incidence of caries and the bacteria found in the molar teeth of cotton rats. In this study, low levels of sucrose and honey were used as part of the carbohydrate portion of purified rations. The incidence and extent of caries and the corresponding bacterial counts were determined. The results of these quantitative studies and the identification of the predominant microorganisms obtained from carious teeth are reported in this paper.

EXPERIMENTAL

A method has been described for making quantitative bacterial counts on teeth of the cotton rat.[6] In brief, this method consisted of extracting, weighing, and grinding the teeth and making quantitative serial dilution counts. The bacterial counts were calculated on the basis of numbers of organisms per gram of tooth.

This method was used to make bacterial counts on teeth extracted from several groups of animals which had been fed experimental rations. Weanling cotton rats were used for these experiments. The animals were kept on experiment for fourteen weeks.[7] Animals from each litter were distributed equally among all experimental groups since a strain variation in caries susceptibility has been observed.[8] At the end of the experimental period the animals were killed and bacterial counts made by the method described. The incidence and extent of carious lesions in the remaining three quadrants were determined by the method of Shaw, Schweigert, McIntire, Elvehjem, and Phillips.[7]

The experimental rations used consisted of 24 per cent vitaminized casein, 67 per cent carbohydrate, 5 per cent corn oil, and 4 per cent salts. Variations

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were made in the carbohydrate portion of the diet in the following manner: sucrose or honey was fed at levels of 2, 5, and 10 per cent of the entire ration with the balance of the carbohydrate being floured dextrin.

Since the stock diet is known to be a low caries-producing diet, animals on stock ration were used as controls. Bacterial counts and caries scores on the teeth of these rats were determined as before.

The organisms predominant in the ground teeth were isolated from the carrot-liver agar shake tube cultures. Gram stains of the various cultures were made to determine purity and morphological characteristics of the organisms. The ability of the cocci obtained to grow in 6.5 per cent sodium chloride broth at 37° C. and in sodium azide broth at 45° C. was determined. Action on litmus milk and ability of these cocci to reduce 0.1 per cent methylene blue milk were noted. In this manner enterococci were separated from other lactic acid-producing cocci. The lactobacilli were identified by the usual means. As nearly as possible the cultures were representative of all groups of experimental animals.

RESULTS

The average incidence and extent of carious lesions, and the corresponding shake tube and plate counts for each group of animals, are shown in Table I. Animals fed the stock diet were found to have few, if any carious lesions. Low bacterial counts were obtained from the ground teeth of these animals.

TABLE I

AVERAGE INCIDENCE AND EXTENT OF CARIOUS LESIONS AND CORRESPONDING BACTERIAL COUNTS

RATION	AVERAGE INCIDENCE*	AVERAGE EXTENT†	AVERAGE COUNTS PER GRAM OF TOOTH IN CARROT-LIVER SHAKE TUBES	AVERAGE COUNTS PER GRAM OF TOOTH IN NUTRIENT AGAR PLATES
857 2% sucrose	7.2	11.3	177,540,000	13,030,000
858 5% sucrose	8.7	17.9	147,570,000	10,860,000
859 10% sucrose	9.5	20.7	193,510,000	5,670,000
860 2% honey	15.1	36.6	290,640,000	20,060,000
861 5% honey	22.8	67.1	350,030,000	23,580,000
862 10% honey	13.9	33.8	245,400,000	10,200,000
Stock ration	0	0	14,410,000	7,526,000

*Number of carious lesions per head.
†Extent of carious lesions per head.

No appreciable differences, either in incidence and extent of carious lesions or in bacterial counts, were obtained when the levels of sucrose in the ration were varied from 2 to 10 per cent. However, an increase in the incidence and extent of caries and bacterial shake tube counts resulted when honey at 2 to 10 per cent replaced sucrose in the cotton rat's diet. Ten per cent honey failed

to produce greater incidence and extent of caries than 2 per cent honey. It may be noted that there were no significant differences in the nutrient agar plate counts as the extent of carious lesions increased.

Organisms were isolated from carrot-liver agar shake tube cultures made from ground teeth. Preliminary identification indicated that the organisms from the ground tooth cultures fell into two main groups: enterococci and lactobacilli (Table II). Of particular interest was the strong proteolytic action of the enterococci on litmus milk. In eighteen hours, acid was produced and the litmus partially reduced, but no curd was formed. Proteolysis was partial at forty-eight hours and complete in seventy-two hours. These organisms closely resemble *Streptococcus liquefaciens*. Of sixty-five cultures isolated from ground teeth, 77 per cent were identified as enterococci and 16 per cent as lactobacilli.

DISCUSSION

It as been shown that in human subjects an increase in numbers of microorganisms occurs when the incidence of caries increases. Harrison[9] has shown that the relationship of the buccal flora of rats to caries is similar to that in man. Orland,[10] investigating the oral flora from the molar tooth surfaces of the Syrian hamster, has found that comparison of caries indices with the number of organisms indicates some degree of association of lactobacilli and streptococci with the development of carious lesions.

TABLE II

CULTURAL CHARACTERISTICS OF ORGANISMS ISOLATED FROM GROUND TEETH

MORPHOLOGY	NO. OF CULTURES	ACTION ON LITMUS MILK			GROWTH IN PRESENCE OF 6.5% NaCl IN BROTH	GROWTH IN 0.1% METHYLENE BLUE MILK	GROWTH IN SODIUM AZIDE BROTH AT 45° C.	GRAM-STAIN REACTION
		ACID	STRONG REDUCTION	PROTEOLYSIS				
Cocci in pairs or short chains	39	+	+	+	+	+	+	+
	11	+	slight	+	+	−	+	+
	1	+	+	+	+	−	−	+
	1	+	slight	−	−	+	+	+
Long granular rods	6	+	slight	−	−	−	−	+
	6	+	+	−	−	+	−	+
	1	+	+	+	−	−	−	+

In this study the correlation between the incidence and extent of caries and the carrot-liver agar shake tube culture counts is believed to be significant.

No appreciable difference in the incidence or extent of caries, or in the corresponding bacterial counts, was obtained when the levels of sucrose or honey were varied from 2 to 10 per cent; this is probably due to the low levels of the sugars used.

Both lactobacilli and enterococci were found in the cultures isolated from ground carious teeth of the cotton rat. However, the enterococci were found to be more numerous than the lactobacilli in these cultures. As mentioned previously, the enterococci isolated were found to be strongly proteolytic. It is particularly interesting to note in connection with this finding that Gottlieb[11] and Frisbie, Nuckolls, and Saunders[12, 13] have described proteolysis as an important factor in the carious process.

SUMMARY

Correlation was obtained between the incidence and extent of carious lesions and the bacterial counts of teeth from cotton rats fed diets containing low levels of honey and sucrose. The incidence and extent of caries, and the corresponding bacterial counts, were much greater when honey replaced sucrose at similar levels (2 to 10 per cent) in these rations. No significant difference could be noted either in incidence and extent of carious lesions or in bacterial counts when the levels of sucrose or honey in the ration were varied from 2 to 10 per cent.

Organisms isolated from carious teeth were identified as lactobacilli and enterococci. Approximately four times more enterococci than lactobacilli were found in carious teeth.

The strong proteolytic action of the enterococci found tends to emphasize the importance of organic matrix of the tooth and its relationship to dental caries.

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PAPER PARTITION CHROMATOGRAPHY: FREE AMINO ACIDS IN SALIVA

A Preliminary Report

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INTRODUCTION

The amino acid content of the saliva has been the subject of a series of investigations by Kesel and his collaborators[1-3] designed to clarify the source of salivary ammonia. Utilizing microbiological technics they showed the presence of at least eleven amino acids in whole saliva of three groups of subjects classified according to caries activity. They concluded that the source of ammonia is not entirely urea but that the amino acids (tryptophane, arginine, isoleucine, serine, valine, glutamic acid, phenylalanine, threonine, lysine, glycine, and tyrosine) also contribute to its formation. No apparent correlation was found between the amount of these substances present in the saliva and caries activity.

Kirch, Kesel, O'Donnell, and Wach,[4] utilizing the same technics, have shown the presence of sixteen amino acids in saliva. In addition to the eleven previously found,[3] the following were reported: proline, leucine, cystine, histidine, and methionine.

Some workers have concentrated their attention on the presence of one amino acid. For example, utilizing a chemical reaction, Turner and Crowell[5] have attempted to show an inverse relationship between the presence of tryptophane and the breakdown of starch. They have concluded that there is an inverse correlation between the amount of tryptophane found in saliva and the caries activity index of the individual.

Dent[6] has demonstrated the many useful applications of paper partition chromatography in the investigation of the amino acids of blood and urine. The idea suggested itself to the authors that this technic might be applied to the study of the amino acids of the saliva.

METHODS

The procedures and apparatus employed in paper chromatography are of the utmost simplicity. Two-dimensional chromatography utilizes a sheet of filter paper (18 by 22 in.) near the upper corner of which is placed a small quantity of sample. The upper edge of the paper is dipped into a trough containing water-saturated phenol and the solvent is allowed to flow down the paper by capillary action. The phenol is then evaporated off, the paper rotated

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90 degrees, and the procedure repeated with collidine as solvent. The amino acids (and peptides) are thereby carried across the paper in a characteristic fashion. After application of a ninhydrin solution, the amino compounds appear as colored spots and are identified by their relative positions on the paper. As little as 1 microgram of acid is sufficient for detection. One-dimensional chromatography employs a long, narrow strip of paper and but one solvent. It is used as a check upon the amount of unknown before the more time-consuming two-dimensional analysis is undertaken. The reader is referred to the literature[6, 7] for a complete discussion of the theory and practice of this technic.

All saliva was collected at 11:00 A.M., flow being stimulated by the chewing of paraffin. An interval of two hours had elapsed since the ingestion of the last meal. Smoking had been curtailed for at least one hour previously. The collection was continued until the subject contributed approximately 20 ml. of saliva. This usually took from 20 to 30 minutes. The recommendation of Deakins[8] being accepted, the saliva was collected in a test tube thoroughly immersed in ice, thus reducing to a minimum changes in the salivary protein. All samples were pooled immediately upon collection. No attempt was made to classify the individuals according to caries activity.

The saliva was promptly transferred to cellophane bags, 5 in. long and having a capacity of approximately 30 milliliters. Ordinarily only 15 ml. of saliva was placed in each bag. These bags were an integral component of an ultrafiltration system which utilized nitrogen as the driving force (150 mm. of Hg). The procedure was carried out in a cold room (6° C.) over a period of twenty-four to thirty hours, in which time approximately half of the fluid was forced through the Cellophane. This technic has proved effective in separating crystallites from the proteins and other colloidal material of biological fluids. The filtrate was subjected to the biuret test, and of three tests, one was slightly positive. (The positive nature of the one test does not necessarily indicate that proteins escaped into the filtrate, inasmuch as histidine, serine, and threonine are reported to give positive reactions.)

Five to ten milliliters of the ultrafiltrate were poured into large watchglasses which were placed in a vacuum desiccator over sulfuric acid. After forty-eight to seventy-two hours in the cold room, the material was completely dehydrated. On each run a portion of dried ultrafiltrate equivalent to 5 ml. of whole saliva was dissolved in a minimum quantity (100 microliters) of distilled water and placed on the filter paper.

RESULTS

The results of three chromatographs are presented in Table I. The presence of serine in the third run and histidine in the second run may be questioned as they did not appear in any of the other runs. The significance of "under-alanine" has been partially clarified by Dent[6] who considered it to be a peptide, serylglycylglycine. A number of other peptides appeared in all runs but were not subjected to further analysis.

TABLE I

FREE AMINO ACIDS OF THE SALIVA

AMINO ACID	RELATIVE CONCENTRATION		
	RUN NO. 1	RUN NO. 2	RUN NO. 3
1. Glutamic acid	6x	8x	4x
2. Aspartic acid	3	6	6
3. Glycine	3	8	4
4. Alanine	1	5	5
5. Methionine (Sulfoxide)	3	1	8
6 Arginine	1	3	4
7. Leucine	2	4	3
8. Lysine	2	2	4
9. Phenylalanine	4	2	2
10. Taurine	1	-	4
11. Tyrosine	1	2	3
12. Valine	1	2	2
13. Proline	-	1	1
14. "Under-alanine"	-	4	4
15. Serine	(Present only in the third run)		
16. Histidine	(Present only slightly in the second run)		

None of the other amino acids were found.

The estimation of relative concentration of amino acids was based on the strength of the ninhydrin reactions. These were compared against a color standard twenty-four hours after application of the ninhydrin solution, at which time maximum color saturation is evident. The results are semiquantitative, 1x approximating 1γ and 10x representing a minimum of 100γ of amino acid. The estimates so obtained are accurate within $\pm$ 30 per cent.

DISCUSSION

One great advantage of the paper partition chromatography technic is that it delineates specifically the free amino acids in ultrafiltrates of saliva, and does so without the interference of other nitrogenous substances, in particular, peptides. Previous methods have not necessarily been able to do this and have failed to distinguish free amino acids from those arising from hydrolysis of the salivary proteins or peptides. Further work is contemplated with regard to amino acids present after various hydrolytic technics.

SUMMARY

Paper partition chromatography technic applied to ultrafiltrates of pooled saliva showed the presence of thirteen free amino acids in saliva. The acids found were: glutamic acid, aspartic acid, glycine, alanine, methionine (sulfoxide), arginine, leucine, lysine, phenylalanine, taurine, tyrosine, valine, and proline. The method is also capable of demonstrating peptides, one of which was probably serylglycylglycine.

The technic has distinct advantages over methods previously used, in that all the amino acids present in saliva can be identified without interference of peptides or other nitrogenous products.

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THE EFFECT OF AN ACIDULATED FLUORIDE MOUTHWASH ON DENTAL CARIES

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THE demonstration[1] that a reduction of approximately 40 per cent in the occurrence of new caries resulted from making three topical applications per year of a fluoride solution containing as little as 0.1 per cent sodium fluoride suggested to us that more frequent applications of even more dilute fluoride solutions such as could be incorporated in a mouthwash might have a similar caries-reducing effect. That low concentration of fluoride might confer an added caries resistance to the teeth is indicated by evidence[2] that fluoride concentrations as low as one part per million in drinking water may act topically to reduce caries. That only a brief contact between fluoride solutions and the teeth is needed is suggested both by laboratory observations and by the finding that fluoride treatments give smaller caries reductions between treated and untreated quadrants in lower than in upper teeth. This is apparently the result of a greater leakage of fluoride to the untreated teeth of the lower jaw during the period of treatment. Bossert and Dunning[3] have reported that fluoride solutions maintain an effective concentration in the mouth when used as a mouthwash. These considerations made it seem worth while to test again the possibility of reducing caries by the use of a fluoride-containing mouthwash.

Two reports on the use of fluoride mouthwashes have been published. In one, Atkins[4] reported a reduction of caries activity after using water containing up to 0.0004 per cent fluorine. Unfortunately, no direct clinical data are presented on the twenty-five uncontrolled patients tested, and his tentatively positive conclusions are based on a reduction in the lactobacillus count alone. In the other study,[5] which was also of a preliminary sort, three groups of dental students used an acidulated mouthwash containing 0.01 per cent sodium fluoride, a corresponding nonfluoride mouthwash, or no mouthwash. No reduction in caries activity was found in the thirty-one students who could establish satisfactory use of the fluoride mouthwash for one year. This study left doubt as to whether the negative findings were the result of the small size of our experimental group, of the adult age of the experimental subjects, or whether it indicated a general ineffectiveness of mouth washing as a method of fluoride therapy, or merely that our particular mouthwash mixture was of itself ineffective.

In an attempt to answer more conclusively the question of the possible effectiveness of a fluoride mouthwash, a study was organized among the sixth grade children of twelve Massachusetts schools. An examination of the teeth of

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more than 500 children was made by means of a mirror and explorer and the data on caries incidence recorded in a way which would enable it to be transferred to punch cards for machine sorting. On the basis of the findings in this dental examination, the schools were paired so that they fell into two groups, each containing more than 250 children which matched each other as far as social and economic conditions and caries activity were concerned.

Because it was the feeling of the authors that under the usual conditions of home life children the age of our study group would not use mouthwash more frequently than once or twice a week, we avoided setting up an experiment requiring an artificially high frequency of mouth washing. It was our feeling that in the absence of close supervision any therapeutic procedure which depended upon daily or nearly daily use by children was almost certainly going to fail. Accordingly, the mouthwashes were used only twice weekly.

The experimental mouthwash used contained 0.01 per cent sodium fluoride acidulated to pH 4.0. The control mouthwash was the same except that the fluoride was omitted. These mouthwashes were prepared according to our specifications by the Upjohn Company of Kalamazoo, Michigan, and sent by them to the designated schools.

The mouth washing was carried out first under the direct supervision of the authors and subsequently under supervision of the school teachers. In the early part of the morning session the children, who had been instructed to present themselves with well-brushed teeth, were arranged in line and given mouthwash in paper cups at one point in the school building. Tooth rinsing was begun immediately and continued until the line of children reached a sink where the mouthwash could be expectorated. In this way it was possible to insure that the children held the mouthwash in their mouths for a period of at least one minute. During the summer vacation period, each child was supplied with an individual bottle of mouthwash and given suitable instructions regarding its continued use. Neither the teachers in the schools nor the students knew whether a fluoride-containing or a fluoride-free mouthwash was being used. The mouthwashes were indistinguishable in taste and pleasant enough in flavor that both were in high demand by those not required to participate in the experiment.

After a period of one year of mouthwash use, the participating children were re-examined. Unfortunately, it was necessary to use a different examiner from the one who made the first examination. The second examiner had no knowledge of which children were using the fluoride mouthwash. In about 2 per cent of the recordings the interpretations of the original or the second examiner were open to question. Such recordings are excluded from our tabulations. At the end of the one-year period, only 187 of the children who had consistently used sodium fluoride mouthwash and 169 of the children using the control mouthwash were available for re-examination. A summary of the findings is presented in Table I.

It is obvious that the fluoride-containing mouthwash did not bring about a reduction in caries activity. On the other hand, it appears that the caries

TABLE I

CARIES ATTACK IN PERMANENT TEETH OF CHILDREN USING MOUTHWASHES

MOUTHWASH	NO. OF PATIENTS	SOUND TEETH AT BEGINNING OF STUDY	NEW D F TEETH	ATTACK RATE (TOOTH) (PER CENT)	SOUND SURFACES AT BEGINNING OF STUDY	NEW D F SURFACES	ATTACK RATE (SURFACE) (PER CENT)
Fluoride	187	2,593	256	9.9	16,429	564	3.4
Control	169	2,397	177	7.4	14,730	409	2.8

activity was actually higher in the children using the fluoride mouthwash than in those using the control mouthwash, there being an increase of approximately 30 per cent in the tooth attack rate and about 20 per cent in the surface attack rate in the former group.

COMMENT

The finding that there was no reduction in caries activity as the result of using the fluoride mouthwash is not too surprising, but the discovery that its use apparently increased caries activity demands explanation. On one hand, we have laboratory evidence[6] that acidulation of fluoride solutions increases their capacity for protecting enamel against acid decalcification. On the other hand, the available clinical evidence does not show that acidulated fluorides have an increased effectiveness in reducing the activity of dental caries. Neither this study nor the published reports (Arnold, Dean and Singleton,[7] and Bibby, Zander, McKelleget, and Labunsky[5]) on the clinical use of acidulated solutions of sodium fluoride show a reduction of caries. We[8] also have evidence that topical applications of acidulated lead fluoride solutions are less effective than neutral solutions in combating caries. In addition, animal experiments[9] have actually revealed a caries increase after-topical applications of acidulated sodium fluoride. In short, all efforts to reduce caries by using acidulated fluorides have failed.

Speculation as to how this apparent reversal in the effect of sodium fluoride is brought about is complicated by the absence of definite knowledge of the way fluorides react with the teeth to make them resistant to dental decay. An explanation may be that in our laboratory studies where we used fluoride buffers of a pH lower than the iso-electric point of enamel, there may have been active decalcification followed by adsorption of calcium fluoride or some other fluoride compound on the enamel which then served to protect the enamel surface from the action of acid. It is possible that if in our laboratory studies the pH of the system had been raised to the other side of the iso-electric point, the adsorbed material might have been released, leaving an unprotected enamel surface. We did not test the possibility. In the mouth, fluorine compounds adsorbed during the treatment with the acidulated fluoride would be lost as soon as saliva gained access to the teeth and restored the pH to its normal level. If such changes do occur in the mouth, we would have not only the immediate removal of the fluoride-protecting factor which modified the test tube results

but also the loss, through decalcification, of a microscopic layer of the original tooth surface which may have previously acquired a surface acid resistance of some other sort.

SUMMARY

The effect of a pH 4 mouthwash used twice a week was tested for one year on sixth grade children. The 187 children using an acidulated sodium fluoride mouthwash had a caries attack rate more than 30 per cent higher than 169 children using an acidulated fluoride-free mouthwash. It is suggested that this negative effect of fluoride treatments may be the result of acidulation of the mouthwash used and an explanatory hypothesis is offered.

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HOW ABRASIVE NEED A DENTIFRICE BE?

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THE outstanding advantage of a liquid dentifrice is its nonabrasiveness. This characteristic has been demonstrated by several investigators.[1, 2, 4]

The outstanding disadvantage of the liquid dentifrice is that an appreciable percentage of persons develop a visible brown to black stain on the teeth while using it. Such a condition was noted by one of us (P. C. K.), as early as 1938 while working with a new liquid dentifrice with a group of dental students used as test subjects. It was observed that about 16 per cent of a group of sixty students had readily visible tooth staining after using a 2.5 per cent sodium lauryl sulfate liquid dentifrice. It was also noted that this stain did not occur on porcelain restorations and did not react to iodine disclosing solution in the the manner of a carbohydrate.*

The first publication on this stain which accompanies the use of liquid dentifrices was by Manly[3] in 1943. Later, Vallotton[5] made an extensive study of this "brown pellicle." His estimate that the teeth of over 60 per cent of persons stained with a nonabrasive dentifrice was an estimate of all stain to be seen even with a binocular microscope, not merely stain visible to the unaided eye.

In a number of projects carried on at the Ohio State University it has been necessary to have heavy and photogenic "stainers." In screening dental students to find good stainers (testing with brush and water for two weeks, following a thorough stain removal) for the present project, we examined 113 individuals and found only twenty (18 per cent) whose staining could be classified as heavy and obvious to the naked eye. On the other extreme, we found only five (4 per cent) who were free from any stain when examined with the binocular microscope. There were twenty-four (21 per cent) whose stain was not obvious to the naked eye but could be seen on one or more teeth through the binocular microscope. The remaining sixty-four (57 per cent) were intermediate in their staining reactions between the heavy stainers and those whose stain was so slight on all teeth as to be invisible to the unaided eye.

Vallotton[5] has also reported that this stain occurred when water was used as a dentifrice by six individuals who had previously stained with a liquid dentifrice. For a number of years we have screened dental students for staining reaction by the use of brush and water. It is quite evident that this stain is not a peculiar property of any liquid dentifrice but that it is merely something that a nonabrasive dentifrice does not remove. As reported by Manly,[3] one or two applications of abrasive dentifrice usually will remove the stained pellicle.

The whole question of dentifrice abrasiveness has been brought into sharper focus by this stained pellicle. That there is a great variation in the ability of current commercial dentifrices to cut into the cervical areas of teeth (abrasive-

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ness) has been shown by Kitchin and Robinson.[6] That many individuals require some abrasiveness in dentifrices if they are to remain stain free is generally concluded as a result of the performance of liquid (nonabrasive) dentifrices. The important question is, "How abrasive need a dentifrice be?"

In the light of the fact that the only recognized function of a dentifrice is to aid the brush in cleaning the accessible tooth surfaces, it would seem reasonable to answer that a dentifrice should be just abrasive enough to cause the subject's teeth to remain free from stain. It should be no more abrasive, however, since any loss of cementum or dentin on exposed cervical areas is undesirable.

We have reported on the abrasiveness of twenty-one dentifrices ranging from water, with practically no abrasive action when used on the cervical area of a tooth, to a commercial tooth powder capable of cutting a notch four and one-half millimeters deep in 100,000 cross strokes. Our purpose in computing this graded series was to be able to test each dentifrice on a group of heavy stainers so that we could correlate the abrasion factor of each with its ability to prevent visible stain accumulation. The ability of a dentifrice to prevent the accumulation of a stained pellicle on the teeth was determined through its use by individuals who had shown themselves to be heavy stainers when they used a brush and water for a two-week period.

EXPERIMENTAL PROCEDURE

As a result of screening 113 dental students, we selected twenty relatively heavy stainers, fifteen of whom constituted the original test group. Each test of a dentifrice on this group occupied a two-week period; (eighteen of the dentifrices and salt and soda were tested. In addition, basic stain tests on brush and water were conducted. The original fifteen stainers were used for the first five test periods. At the end of this time three were lost to the test group by graduation. For the succeeding nine test periods the remaining twelve individuals constituted the test group of stainers. At the end of that time seven more graduated. The group of five remaining stainers was then augmented by the unused five of the original pool of twenty. This group of ten was used for all the remaining stain tests.

Whenever individuals left the test group, or were introduced into it, they were checked for staining by a two-week period on brush and water. This assured us that they had not changed from stainers to nonstainers during their time on the tests or while awaiting entrance into the test group. For those entering the test group late, the more recent stain score on water was used for comparison with results on various dentifrices.

In every test, stainers started with clean teeth and a new professional model medium nylon bristle toothbrush. There was no attempt to influence the individual's method of tooth brushing.

At the end of each test period the amount of stained pellicle on each of the twelve anterior teeth (six upper and six lower) of each test subject was determined independently by each examiner (H. B. G. R. and P. C. K.). There was a surprising degree of uniformity on these scores. Wherever a marked variation occurred a re-examination was made and mutually satisfactory results were secured.

Stain scoring was done with a binocular microscope and good artificial light on the teeth. If no stained pellicle could be seen by scraping a tooth surface with a small sharp-edged dental instrument, the score was zero. If it could be seen only when scraping and the binocular scope was employed, the score was 0.5 (also indicated as ±). If the stain was visible to the naked eye, it was scored as 1 to 4, depending on the thickness and visibility of the pellicle.

The scoring of the stain (in the range of 1 to 4) was necessarily a matter of personal judgment. However, the questions of whether it was absent or just visible with scraping using the binocular microscope, and whether or not the stain was visible to the naked eye, were not so much matters of opinion. The fact that all scoring represents the judgment of the same two independent observers gives, we believe, comparative figures of fair reliability. At the end of each test period, Kodachromes were made by the teeth of each student cooperator. This was visual evidence of stain.

RESULTS

From the individual stain scores of each tooth, the percentage of all teeth with visible stain at the end of each dentifrice test period was determined. The use of mere visibility stain, rather than any definite degree of visibility, removed the question of judgment as to whether the degree of stain was 1, 2, 3, or 4. If it was visible it was objectionable; and if a greater percentage of all the teeth were visibly stained, the condition was more objectionable than if a lesser percentage showed visible stain.

TABLE I

SUMMARY OF DATA ON ABRASION AND STAIN TESTS

DENTIFRICE	DENTIN CUT (MM. PER 100,000 STROKES)	AVERAGE STAIN SCORE	PER CENT OF COOPERATORS WITH ONE OR MORE TEETH WITH STAIN SCORE OF 1 OR MORE	PER CENT OF ALL TEETH WITH STAIN SCORE OF 0 OR ± (STAIN NOT VISIBLE TO NAKED EYE)	PER CENT OF ALL TEETH WITH STAIN SCORE OF 1 OR MORE (VISIBLE STAIN)	SAFETY INDEX*
Water	0.006 ± .0056	25.3-26.9	100.0	8.4	91.6	1400
4699 B	0.12 ± .0200	10.6	93.3	49.7	50.3	414
4780	0.24 ± .0380	5.1	58.3	81.8	18.2	341
4781	0.26 ± .0405	No test				
Colgate paste	0.31 ± .0334	4.9	53.3	81.6	18.4	263
Salt and soda	0.31 ± .0479	No test				
4911 B	0.41 ± .0316	4.4	41.7	84.6	15.4	206
4680 B	0.42 ± .0562	5.7	71.0	76.7	23.3	182
5079	0.48 ± .0767	3.6	30.0	92.5	7.5	193
4660 B	0.56 ± .0915	6.0	66.7	78.8	21.2	141
Pepsodent paste	0.56 ± .0690	3.3	41.7	90.9	9.1	162
4774	0.61 ± .0245	5.8	70.0	81.5	18.5	134
Pycopay	0.67 ± .0318	5.5	66.0	79.7	20.3	119
4770	0.86 ± .0435	4.7	58.3	83.9	16.1	97
Listerine paste	0.98 ± .1727	2.1	26.6	96.0	4.0	98
4700 B	1.07 ± .0890	3.9	41.7	86.7	13.3	81
Ipana paste	1.25 ± .0898	2.0	40.0	95.0	5.0	76
Colgate powder	2.02 ± .3115	0.9	20.0	98.3	1.7	49
Pepsodent powder	2.26 ± .188	0.6	0.0	100.0	0.0	44
Calox powder	3.23 ± .868	No test				
Dr. Lyon's powder	4.48 ± .553	0.6	8.3	99.3	0.7	22

*Safety Index = $\frac{\%\text{ of teeth with scores of 0 or } \pm}{\text{mm. of dentin cut per 100,000 strokes}}$

Table I gives a summary of the abrasiveness and stain-removing ability of each dentifrice.

The average stain scores (Column 3), while significant for dentifrices of widely varying abrasive qualities, fail to correlate with small differences. They also fail to give any data as to the distribution of the stain scores on which they are based.

The percentage of cooperators with one or more teeth with stain visible to the naked eye (stain score 1 or more, Column 5) shows correlation with dentifrice abrasiveness only in a general way. It should be noted that an individual could be put in this category as a result of one lingually displaced tooth which was relatively inaccessible to the brush. This column, as well as the average stain scores, brings out some of the weaknesses of our system of stain scoring.

Columns 5 and 6 are complementary and, we believe, more significant. They take into account all the teeth scored, and differentiate between teeth with stain visible to the naked eye and teeth with no stain, or not enough to be evident to the naked eye.

In order to correlate abrasive effects and cleaning ability we have constructed a curve (Fig. 1) using the percentages of teeth with stain visible to the naked eye (stain score of 1 or more) on the ordinate and the dentin cut per 100,000 strokes on the abscissa.

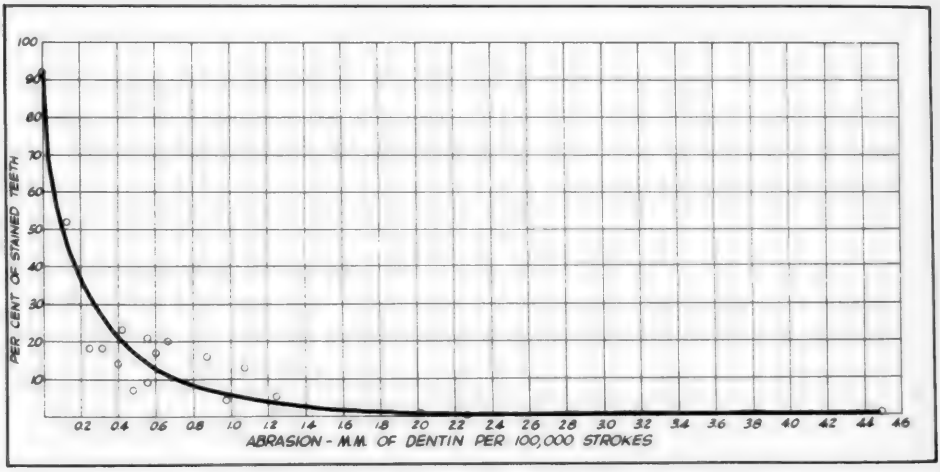

Fig. 1.—Curve showing relationship between dentifrice abrasiveness on dentin and ability to remove stained pellicle from the enamel surface.

From such a curve it is apparent that stain-removing ability is generally in direct relationship to the ability to abrade dentin. More specifically, however, there is a sharp drop in the percentage of visibly stained teeth as the abrasiveness rises from 0.0 to 0.6. This drop is less from 0.6 to 1.0, but still marked. Beyond an abrasiveness of 1.0, any cleaning ability (and there does not seem to be much) is purchased at the cost of an excessive loss of cementum and dentin.

If we may consider the safety of a dentifrice on the basis of the number of stainers' teeth maintained free of visible stain per millimeter of dentin abrasion, we can compute the safety index by dividing the number of teeth with ± or 0 scores by the dentin cut per 100,000 strokes of the dentifrice concerned. This is given in Column 7 of Table I. An individual with cervical exposure should, we believe, use a dentifrice with as high a safety index as is consistent with the inhibition of visible stain. Practically, this indicates that nonstainers may use nonabrasive dentifrices with entirely satisfactory results. A stainer will require some degree of abrasive action to prevent the accumulation of stain. The least abrasive dentifrice which will present stain accumulation is the most desirable; it might be determined by trial on any individual. Until some nonabrasive, stained pellicle-removing agent suitable for use in a dentifrice is found, as high as 50 to 75 per cent of the population will require some degree of abrasiveness in their dentifrices. Dentifrice abrasion should be considered as a necessary evil to be avoided as much as possible, even in this group of the population, by the intelligent selection of a dentifrice.

From the data in Table I and Fig. 1, it appears that one commercial paste and four commercial powder dentifrices purchased in 1946 were unnecessarily abrasive, even for heavy stainers (with cervical exposure); i.e., they cut more than 1 mm. of dentin per 100,000 strokes under test conditions without possessing special stain-removing properties justifying this abrasiveness.

Reliable current information on dentifrice abrasion, as determined on cervical areas of cementum and dentin, should be available to the public as long as some abrasiveness is considered a necessary or desirable characteristic of a dentifrice.[7] In view of our experience, we believe such data, to be valid, should be secured by *testing dentifrices on dentin*, and not by tests on the abrasive agents alone.

SUMMARY

Stained pellicle is not produced by liquid dentifrices, but results from accumulation of stain on the teeth of certain individuals using water or other nonabrasive dentifrices. The degree of staining is an individual characteristic.

Using groups of dental students who were heavy stainers with brush and water, we determined staining of their teeth while they were using a series of commercial dentifrices of different degrees of abrasiveness. These dentifrices varied from water, which is practically nonabrasive when applied with cross-brushing to the cervical areas of teeth, to a commercial tooth powder capable of cutting a notch 4½ mm. deep with 100,000 strokes.

Dentifrice abrasiveness greater than that necessary to cut 1 mm. per 100,000 strokes into the cervical area of teeth with the apparatus employed, appears to be unnecessary even for very heavy stainers who would not constitute more than 20 per cent of a population. Only one of the commercial paste dentifrices tested was excessively abrasive for heavy stainers. The commercial powders tested, with one exception, were from 100 per cent to 300 per cent more abrasive than was found necessary to accomplish stain prevention in our tests on heavy stainers.

Generally speaking, for stainers with cervical exposure, a dentifrice with a safety index as high as is consistent with prevention of stain accumulation is desirable. Such a dentifrice should be determined on an individual basis.

Dentifrice abrasion, for obvious stainers, will be a necessary evil until a nonabrasive, stain-removing or preventing agent is found. Reliable current information on dentifrice abrasion, determined by testing whole dentifrices on dentin, should be available.

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PARTICLE SIZE DETERMINATION WITH THE AIR PERMEABILITY METHOD

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A GREAT number of methods, based on a variety of principles, have been used for the determination of the size of small particles. Such diversified methods as microscopic measurement, sedimentation techniques, centrifugal analysis, elutriation with air or liquids, turbidimetry, absorption and permeability have been employed successfully in the study of small particles.[1]

The object of the present investigation was to select a method adaptable to the routine determination of the particle size of powders in the micron and submicron range.

APPARATUS

Recently, an apparatus employing the air permeability principle has been placed on the market.*† This instrument is a development of the original design by Gooden and Smith,[2] and based on the work of Carman,[3, 4] Traxler and Baum,[5] Dalla Valle,[6] and Lea and Nurse[7] which showed that a definite relationship existed between the permeability of a packed column of a powder and its specific surface. The average particle diameter estimate obtained by this method is the diameter of a sphere having the same specific surface, that is ratio of surface to volume, as the powder.

REPRODUCIBILITY

To test the reproducibility of this Fisher instrument, four powders of different estimated particle size and different chemical composition were selected and measured on different days. Table I shows the values obtained. From the very small standard deviations it can be seen that this instrument gives reproducible and consistent results.

COMPARISON WITH PREVIOUS METHODS

In addition, measurements were carried out on a number of dentifrice powders which had been previously studied by Tainter and co-workers. The values obtained for these materials by the air permeability method are compared, in Table II, with the values previously reported by Tainter, Epstein, and Klein[8] for these same samples, using microscopic and hydrometer methods. For the comparison in Table II, however, the specific surface data obtained by the hydrometer method were used to calculate the diameter of a sphere having the same specific surface as the powder.

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*Fisher Sub-Sieve Sizer, Fisher Scientific Co., No. 14-312.

†U. S. Patent 2,261,802, Nov. 4, 1941.

TABLE I

REPRODUCIBILITY OF PARTICLE SIZE DETERMINATIONS

MATERIAL	DAY TESTED	POROSITY (%)	PARTICLE SIZE (μ) AND STANDARD DEVIATION (δ)
Calcium carbonate	10/ 9/47	54.2	8.5
	10/ 9/47	54.6	8.7
	10/10/47	55.2	8.7
	10/10/47	54.3	8.7
	10/10/47	54.3	8.7
	10/13/47	54.6	8.5
	10/13/47	54.3	8.5
	10/14/47	53.7	8.5
	10/14/47	54.3	8.5
	10/14/47	54.6	8.5
	Average	54.4	8.58 ± .10
Dicalcium phosphate	10/ 9/47	59.7	1.90
	10/ 9/47	59.9	2.06
	10/10/47	63.5	1.80
	10/10/47	63.2	1.80
	10/10/47	62.3	1.80
	10/13/47	58.3	2.02
	10/13/47	57.2	2.02
	10/14/47	59.0	2.02
	10/14/47	59.6	2.02
	10/14/47	59.7	2.02
	Average	60.2	1.95 ± .10
Aluminum hydroxide Batch A	10/ 9/47	71.0	.34
	10/ 9/47	70.4	.36
	10/10/47	71.2	.35
	10/10/47	71.0	.34
	10/10/47	71.3	.36
	10/13/47	70.6	.42
	10/13/47	69.3	.45
	10/14/47	71.6	.35
	10/14/47	70.9	.35
	10/14/47	71.0	.34
	Average	70.8	.37 ± .03
Aluminum hydroxide Batch B	10/ 9/47	68.1	.60
	10/ 9/47	68.2	.63
	10/10/47	69.0	.68
	10/10/47	67.0	.67
	10/10/47	67.0	.69
	10/13/47	66.8	.68
	10/13/47	65.7	.68
	10/14/47	67.0	.69
	10/14/47	69.8	.69
	10/14/47	67.0	.68
	Average	67.5	.67 ± .02

On the whole, the air permeability method yields lower particle diameter readings than the other two methods, the averages being approximately 0.56 of the microscopic and 0.75 of the hydrometer values. With respect to the microscopic technic, this behavior has been observed before in a study of the particle size of zinc oxide pigments,[9] where the diameters obtained by permeability measurements were only 0.43 of the microscopic values.

TABLE II

COMPARISON OF MICROSCOPIC, HYDROMETER, AND AIR PERMEABILITY VALUES IN THE DETERMINATION OF AVERAGE PARTICLE DIAMETERS OF DENTIFRICE POWDERS

TYPE OF DENTRIFICE POWDER	MICROSCOPIC* (μ)	HYDROMETER† (μ)	AIR PERMEABILITY (μ)
Calcium carbonate			
"Snow Top" Regular, A.D.R.	2.8	2.16	1.14
"Snow Top" Extra Heavy, A.D.R.	4.6	3.91	2.19
"Snow Top" Fine, A.D.R.	1.7	1.10	0.83
"Sturge"—70, A.D.R.	2.2	4.43	1.92
"Sturge"—130, A.D.R.	8.2	5.39	5.10
Sedimented, 1-5 μ	3.0	2.87	1.33
Sedimented, 5-15 μ	13.3	7.33	4.78
Sedimented, 15-30 μ	13.0	13.95	9.25
Dicalcium phosphate			
"Vietor" Fine Air Separated through ¾ inch orifice	4.1	2.30	2.06
"Victor"—Dust Air Separated	1.8	.91	1.88
"Monsanto"—Fine Air Separated ⅝ inch orifice	1.3	1.23	1.44
"Victor" Coarse Air Separated ¾ inch orifice	10.8	3.31	3.93
Sedimented, 1-5 μ	2.8	2.61	2.20
Sedimented, 5-15 μ	8.0	6.71	6.40
Sedimented, 15-30 μ	14.8	13.95	9.00

*Mean value from Tainter, Epstein, and Klein.[3]

†Calculated from specific surface data of Tainter, Epstein, and Klein, particle size diameter of sphere having same specific surface as powder. Correlation between microscopic determinations and air permeability, $r = 0.893$; between hydrometer and air permeability, $r = 0.948$.

Two factors inherent in microscopic methods tend to give high values. Particles smaller than 1 μ are difficult to resolve and can possibly be counted but not measured under the microscope. Therefore, an assumption of an average diameter of 0.5 μ for these particles is usually made[3] so that the accuracy of such determinations suffers. Deviation of the particles from spherical shape will also give higher values; on a microscope slide, particles tend to fall on their flattest side so that the diameter corresponds to the maximum rather than the mean projected area of the particle.[4]

Four factors contribute to the deviations to be expected by the hydrometer method. Incomplete dispersion of the sample in the fluid medium will result in faster settling of the agglomerates and consequently an apparently greater proportion of larger particles. Assumptions as to the diameter of the small particles that do not settle in a reasonable length of time must also be made. Both of these factors tend to give results that are high. On the other hand, deviations from spherical shape will slow up the settling rate as Stokes' law is no longer strictly applicable. Agitation, accompanying the insertion and removal of the hydrometer, and convection currents tend to resuspend particles particularly in the range below one micron. Since the first two factors appear to be the dominant ones, the hydrometer method also gives high rather than low values.

Further comparison of the microscopic count method with the air permeability technic carried out in these laboratories on two aluminum hydroxide powders of pseudo-spherical configuration exhibited excellent agreement as shown in Table III.

TABLE III

PARTICLE SIZE OF ALUMINUM HYDROXIDE POWDERS

AIR PERMEABILITY (AV. OF THREE DETERMINATIONS EACH) μ	MICROSCOPIC COUNTS (APPROX. 750 PARTICLES EACH) μ
3.00	2.93
4.99	5.56

EXTENSION OF RANGE

In some cases it was found desirable to extend the range of the particle size analyzer so that particle diameters of less than 1 μ could be determined with greater accuracy. For this purpose the working pressure of the instrument was quadrupled by replacing the 500 mm. water head with a 147 mm. mercury head. A number of samples of aluminum hydroxide of submicron range were measured first at the conventional pressure and then with the working pressure increased fourfold. The particle diameters, as read from the instrument chart using the ×4 head, were found to be approximately 2.5 times larger than the values obtained under normal operating conditions. Our data, given in Table IV, show the readings obtained with the single and quadruple pressure head. In Column 4 the ratios of ×4 head : single head readings are shown, and in Column 5 the particle diameters calculated from the ×4 head readings, employing a factor of 1:2.5 or 0.4, are given. The instrument can, therefore, be used at the higher head of pressure by applying the correction factor to the figures read from the particle size chart or, preferably, a new chart may be constructed for the head of pressure selected where this seems warranted.

TABLE IV

COMPARISON OF PARTICLE SIZE VALUES AS READ AT NORMAL AND FOUR TIMES INCREASED PRESSURES

SAMPLE	NORMAL PRESSURE HEAD (50 G./CM.^{2}) PARTICLE SIZE AS READ FROM CHART (μ)	FOURFOLD PRESSURE HEAD (200 G./CM.^{2}) PARTICLE SIZE AS READ FROM CHART (μ)	RATIO	CALCULATED ×0.4 OBSERVED
1	0.30	0.84	2.80	0.34
2	0.30	0.87	2.90	0.35
3	0.35	0.88	2.51	0.35
4	0.40	0.98	2.45	0.39
5	0.40	0.90	2.25	0.36
6	0.45	1.08	2.40	0.43
7	0.47	1.17	2.49	0.47
8	0.47	1.12	2.38	0.45
9	0.51	1.29	2.53	0.52
10	0.58	1.29	2.22	0.52
11	0.64	1.65	2.58	0.66
Average			2.50	

SUMMARY AND CONCLUSIONS

The air permeability method affords an easy, rapid, and reproducible determination of the average particle diameter of powders, which is particularly

suitable for use in routine control procedures. Values obtained by this method agree well with data obtained by other methods for particle size determination.

An instrument now available on the market which uses the air permeability principle was found useful in the routine determination of particle size diameters of a variety of powders. For particles in the submicron range, a modification is described which increases the reading accuracy of the instrument in this range.

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DENTAL DEFECTS AND PERIODONTAL DISEASE IN EGYPT, 1946-1947*

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INTRODUCTION

IN MAY, 1946, an oral survey of 994 Egyptian fellaheen (agricultural workers) admitted to the Abbassia Fever Hospital, Cairo, Egypt, was initiated. Many of the fellaheen showed evidences of malnutrition, and many were obviously suffering from various chronic diseases indigenous to Egypt. The survey, by necessity, was to be broad and superficial in scope, but with the aim of making it practical and helpful in the correlation of other oral studies reported in the literature.[1-7] It was believed that such a study might reveal some parallel between geographical distribution, caries index, periodontal disease, obvious nutritional deficiency, and amount and type of calculus.

The paramount oral problems were the poor oral health of the vast majority of Egyptians and by what means these conditions could be alleviated or prevented. A pilot-survey showed that an exploratory type of investigation would be most advantageous. The Abbassia Fever Hospital of 1,000 beds was chosen as the study location, for there were ample patients from both Upper and Lower Egypt available. (Fig. 1.) Etiological factors such as dietary, hereditary, and pathological, and specific metabolic disturbances could not be fully investigated because of the lack of equipment and trained personnel. These, however were investigated as far as the means available permitted.

METHODS AND PROCEDURES

The group studied were 994 average Egyptian fellaheen ranging in age from 15 to 55 years. They represented the low income group whose living and general sanitary conditions were comparable. The fellaheen class is numerically the largest class in Egypt. No patient had previous dental treatment or maintained any adherence to oral prophylactic measures such as efficient tooth brushing. The following data were collected and recorded under respective headings:

Personal Information:	Name, age, sex, occupation, birthplace
Chief Complaint (C.C.):	The most prominent symptoms of which the patient complains

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Personal History (P.H.):	Diseases of childhood, other illnesses, injuries (often obscure because of language difficulties and superstitions)
Present Illness (P.I.):	A chronological history of the disorders, onset, course, duration, previous treatment, subjective symptoms (often obscure for same reasons as above)
Laboratory Tests:	Blood examinations, urinalysis, superficial investigations of deficiency status
General Physical Examination (P.E.):	Psychic state, physical conditions, facies, skin, eyes, tongue, lymph nodes, throat, periodontal structures, teeth

Fig. 1.—Geographical distribution.

The teeth and periodontal examinations were made by the author, each examination being done in duplicate. The aids utilized in making the examination were dental mirrors, good light, sharp explorers, and modified Miller millimeter probes. Missing teeth, the cause of which could not be ascertained with certainty, were not considered in the computation of the caries index. The periodontal structures were closely observed and changes from normal classified according to their clinical symptoms as formulated by Goldman.[8] Normal atrophy of the gingivae was problematical and could not be judged with assurance. Patients were grouped with reference to age and region of birth (Table II) under three main divisions:

1. Dental Defects (Tables II, III, IV, VIII, IX)
2. Periodontal Disease (Tables V VI, VII, VIII, IX, X)
3. Amount and Type of Calculus (Tables VIII, IX, X, XI, XII)

TABLE I

THE SCALE OF QUANTITAVE VALUES

0	Normal
1+	0.1-2 mm change—Slight
2+	2-3 mm change—Medium
3+	3 or more mm —Severe

TABLE II

THE DISTRIBUTION BY AGE GROUP AND REGION OF BIRTH

		AGE IN YEARS											
	NO.	15-20		21-25		26-30		31-35		36-40		41-over	
AREA	EXAM.	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
Upper	361	90	24.4	68	18.8	76	21.0	60	16.6	40	11.0	27	7.4
Lower	633	166	26.2	118	18.6	141	22.2	75	11.8	57	9.0	76	12.0
Total	944	256	25.7	186	18.7	217	21.8	135	13.5	97	9.7	103	10.4

The teeth were charted for caries, missing teeth, occlusion, and depth of gingival pocket formation. The examination not only consisted of classifying the type of periodontal disease but also attempted to give a quantitative evaluation of the change. The degree of inflammation and tissue damage was taken as the index of severity of the disease. The degree of change from normal was measured with a millimeter probe and recorded as in Table I. The maximum change around each tooth was determined; the sum of these readings divided by the number of pockets measured yielded the quantitative index for the case in question.

Gingivitis.—Under the term gingivitis, the marginal and hypertrophic types were given separate classification. The symptoms of marginal gingivitis were considered to be red, bleeding, or easily made to bleed engorged marginal gingivae, either localized or of a generalized nature. Hypertrophic gingivitis was diagnosed when the interdental papillae appeared to be affected and enlarged more than the rest of the gingivae. The presence or absence of inflammation was not considered significant in the diagnosis of hypertrophic gingivitis. The degree of change was measured with a millimeter probe and the maximum change around each tooth recorded.

Ulcerative gingivitis or Vincent's infection was classified under gingivitis and recorded as a 3+ rating. Positive diagnosis was made when the gingivae were sensitive to touch and when there were large numbers of fusospirochetal organisms.

Marginal Periodontitis.—Grouped under this heading were those conditions which presented the symptoms of periodontal disease, either local or general in scope. The gingivae present the cardinal symptoms of inflammation, and bleed easily with pus exuding with or without applied pressure. The quantitative index was obtained as described previously for gingivitis.

Periodontosis Syndrome.—Conditions which revealed wandering teeth without primary involvement of the gingivae, or when local conditions obviously produced the accompanying marginal gingivitis, were grouped under the

classification of periodontosis syndrome. No attempt was made to evaluate a quantitative index since the necessary diagnostic equipment was not available. For analysis, these cases were considered severe (3+).

The mouth was also examined with regard to the amount and type of calculus present on the lingual surfaces of the mandibular anterior teeth and the buccal surfaces of the maxillary molars. The presence or absence of calculus was recorded.

FINDINGS

I. *Dental Defects* (Tables II, III, IV, XII)

1. Lower Egypt revealed a higher incidence of caries (1.1 average per person) than Upper Egypt (0.81 average per person).
2. The caries index for the group studied was 0.95 cavity per person.
3. The average number of carious teeth per carious case was 1.36 per person.
4. Carious teeth to be extracted were 0.26 average per person.
5. The number of cases with no caries was 1 per cent higher in Upper Egypt than in Lower Egypt.
6. The number of compound cavities and teeth to be extracted increased with age in contrast to a decrease in simple cavities.
7. Noncarious cases were more numerous in the younger age groups.

II. *Periodontal Disease* (Tables V, VI, VII, XII, XIII)

A. Marginal Gingivitis:

1. There appeared to be no significant difference in geographical distribution.
2. The index of disease was 45.7 per cent.
3. As age increased after 30 years, a sharp transition from marginal gingivitis to the more complex forms of periodontal disease was noted.
4. This type of gingivitis was the most common of periodontal diseases.

B. Hypertrophic Gingivitis:

1. There was no significant difference in geographical distribution.
2. The index of disease was 12.4 per cent.
3. The condition was present in varying degrees in all ages.

C. Ulcerative Gingivitis:

1. Ulcerative gingivitis was found to be 3 per cent higher in Upper Egypt.
2. Age appeared to bear no influence on susceptibility to infection.
3. The index of infection was 5 per cent.
4. The oral sepsis was the dominant feature in all positive cases.

D. Marginal Periodontits:

1. This disease was 2.6 per cent more prevalent in Lower Egypt.
2. The group over 31 years of age exhibited a marked increase.
3. The over-all index was 29 per cent.
4. The quantitative index demonstrated that as age increased tissue damage also increased in magnitude.

E. Periodontosis Syndrome:

1. The geographical analysis, contrary to dental defects and marginal gingivitis, revealed that periodontosis was greater in Upper Egypt by 5.1 per cent.
2. The greatest incidence appeared in the 36 to 40 years age group regardless of geographical location.
3. The over-all average was 5.6 per cent.

F. No Disease:

1. The greater number of cases appeared to be in patients from Upper Egypt, 3.6 per cent in contrast to 1.5 per cent in Lower Egypt.
2. The normal index was 2 per cent.
3. The younger age groups, as expected, revealed more disease-free mouths.

III. *Calculus* (Tables VIII, IX, X, XI, XII, XIII)

1. There was no significant variation in the amount of calculus deposits to geographical location.
2. The type of calculus correlated with region of birth revealed that the supragingival type was more common, 6.6 per cent, in the groups from Upper Egypt.
3. No significant difference was noted between the groups in reference to the incidence of subgingival calculus.
4. A combination of supragingival and subgingival calculus was the most common form as age increased, Lower Egypt exhibiting 5.4 per cent more cases than Upper Egypt.
5. In 8.1 per cent of the cases no calculus was present.

TABLE III

THE RELATIONSHIP OF DENTAL DEFECTS TO AGE AND REGION OF BIRTH

AGE GROUP	NO. EXAM.	NUMBER AND PER CENT OF PERSONS WITH ONE OR MORE DEFECTS									
		SIMPLE CAVITIES		COMP'D CAVITIES		MISSING TEETH*		CARIOUS TEETH TO BE EXTRACTED		CASES WITH NO CARIES	
		NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
Upper Egypt											
15-20	90	48	53.3	21	23.3	51	56.6	6	6.6	18	20.0
21-25	68	39	57.3	18	26.4	45	66.6	9	13.2	5	7.35
26-30	76	48	63.1	21	27.6	54	71.0	6	7.8	1	1.3
31-35	60	24	40.0	15	25.0	60	100.0	3	5.0	3	5.0
36-40	40	9	22.5	12	30.0	33	82.5	18	45.0	1	2.5
41-over	27	15	15.5	21	77.7	21	77.7	33	122.2	3	11.1
Total	361	183	50.6	108	29.9	264	73.1	72	19.9	31	8.5
Lower Egypt											
15-20	166	120	72.2	66	39.7	111	66.8	18	10.8	13	7.8
21-25	118	96	81.3	54	45.7	78	66.1	21	17.7	7	5.9
26-30	141	69	48.9	51	36.1	102	72.3	18	12.7	9	6.3
31-35	75	39	52.0	24	32.0	54	72.0	18	24.0	9	12.0
36-40	57	18	31.5	27	47.3	45	78.9	42	73.6	6	10.5
41-over	76	60	78.9	69	90.7	69	90.7	66	86.8	4	5.2
Total	633	402	63.5	291	45.9	459	72.5	192	30.3	48	7.5

*The reason for missing teeth could not be ascertained with certainty.

TABLE IV

THE RELATIONSHIP OF DENTAL DEFECTS TO REGION OF BIRTH

REGION OF BIRTH	NO. EXAM.	NUMBER AND PERCENTAGE OF DENTAL DEFECTS									
		SIMPLE CAVITIES		COMP'D CAVITIES		MISSING TEETH		CARIOUS TEETH TO BE EXTRACTED		CASES WITH NO CARIES	
		NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
Upper	361	183	50.6	108	29.9	264	73.1	72	19.9	31	8.5
Lower	633	404	63.8	291	45.9	495	72.5	192	30.3	48	7.5
Total	994	587	59.0	399	40.1	759	72.8	264	26.5	79	7.9

TABLE V

RELATIONSHIP OF PERIODONTAL DISEASE TO AGE AND REGION OF BIRTH

Upper Egypt

AGE IN YEARS	NO. EXAM.	NUMBER AND PER CENT WITH PERIODONTAL DISEASE													
		GINGIVITIS NORMAL		MARGINAL		HYPERTROPHIC		ULCERATIVE		MARGINAL PERIODONTITIS		PERIODONTOSIS SYNDROME		TOTAL DISEASED	
		NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
15-20	90	6	6.6	46	51.1	14	15.5	5	5.5	9	10.0	10	9.0	84	93.3
21-25	68	4	5.8	35	51.4	8	11.7	4	5.8	14	20.5	3	4.4	64	94.1
26-30	76	0	0	38	50.0	6	7.8	5	6.5	24	31.5	3	3.9	76	100.0
31-35	60	0	0	20	33.3	4	6.6	6	10.0	25	41.6	5	8.3	60	100.0
36-40	40	0	0	11	27.5	6	15.0	3	7.5	12	30.0	8	20.0	40	100.0
41-over	27	0	0	4	14.8	3	11.1	2	7.3	15	55.5	3	11.1	27	100.0
Total	361	10	3.6	154	42.4	41	11.3	25	6.9	99	27.4	32	8.8	351	97.1

Lower Egypt

15-20	166	8	4.8	98	59.03	33	19.8	9	5.4	14	7.6	4	2.4	158	96.9
21-25	118	1	.8	66	55.9	7	5.9	4	3.3	38	32.2	2	1.6	117	99.8
26-30	141	0	0	82	58.1	21	14.8	6	4.2	32	22.6	0	0	141	100.0
31-35	75	1	1.3	26	34.6	5	6.6	1	1.3	35	46.6	7	9.3	74	98.6
36-40	57	0	0	18	31.6	8	14.2	1	1.7	21	36.8	9	15.7	57	100.0
41-over	76	0	0	11	14.4	9	11.8	4	5.2	50	65.7	2	2.6	76	100.0
Total	633	10	1.5	301	47.5	83	13.1	25	3.9	190	30.0	24	3.7	623	98.4

TABLE VI

RELATIONSHIP OF PERIODONTAL DISEASE TO AGE IN YEARS

AGE IN YEARS	NO. EXAM.	NUMBER AND PER CENT WITH PERIODONTAL DISEASE													
		GINGIVITIS NORMAL		MARGINAL		HYPERTROPHIC		ULCERATIVE		MARGINAL PERIODONTITIS		PERIODONTOSIS SYNDROME		TOTAL DISEASED	
		NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
15-20	256	14	5.4	144	56.2	47	18.3	14	5.4	23	8.9	14	5.4	242	94.5
21-25	186	5	2.6	101	54.3	15	8.0	8	4.3	52	27.9	5	2.6	181	97.3
26-30	217	0	0	120	55.2	27	12.4	11	5.6	56	25.8	3	1.3	217	100.0
31-35	135	1	.74	46	34.6	9	6.6	7	5.1	60	44.4	12	8.8	134	100.0
36-40	97	0	0	29	29.8	14	14.4	4	4.1	33	34.0	17	17.5	97	99.2
41-over	103	0	0	15	14.5	12	11.6	6	5.8	65	63.1	5	4.8	103	100.0
Total	994	20	2.0	455	45.7	124	12.4	50	5.0	289	29.0	56	5.6	974	97.7

Table VII

Qualitative Evaluation of Persons Examined With Gingivitis (Marginal and Hypertrophic Types) and Marginal Periodontitis

Age in Years	No. Exam.	Normal 0		0.1-2 mm (1+)		2-3 mm (2+)		3-more (3+)		Total with Disease	
		No.	%	No.	%	No.	%	No.	%	No.	%
Marginal Gingivitis											
15-20	256	14	5.4	72	28.1	53	20.7	19	7.4	144	56.2
21-25	186	5	2.6	51	27.4	34	18.2	16	8.6	101	54.3
26-30	217	0	0	50	23.0	37	17.0	33	15.2	120	55.2
31-35	135	1	.17	24	17.7	12	8.7	10	7.4	46	34.07
36-40	97	0	0	8	8.2	13	13.4	8	8.2	29	29.8
41-over	103	0	0	1	.97	5	4.8	9	8.7	15	14.5
Total	994	20	2.01	206	20.7	154	15.4	95	9.5	455	45.7
Hypertrophic Gingivitis											
15-20	256	14	5.4	26	10.1	11	4.2	10	3.9	47	18.3
21-25	186	5	2.6	5	2.6	4	2.1	6	3.2	15	8.05
26-30	217	0	0	14	6.4	4	1.8	9	4.1	27	12.4
31-35	135	1	.17	3	2.2	1	.17	5	3.7	9	6.6
36-40	97	0	0	1	1.02	6	6.1	7	7.2	14	14.4
41-over	103	0	0	1	.97	4	3.8	7	6.7	12	1.16
Total	994	20	2.01	50	5.0	30	3.0	44	4.42	124	12.4
Marginal Periodontitis											
15-20	256	14	5.4	6	2.34	3	1.17	14	5.4	23	8.9
21-25	186	5	2.6	1	.5	12	6.4	39	2.09	52	27.9
26-30	217	0	0	6	2.76	10	4.6	40	18.4	56	25.8
31-35	135	1	.17	5	3.7	18	13.3	37	27.3	60	44.4
36-40	97	0	0	1	1.02	14	14.4	18	18.5	33	33.6
41-over	103	0	0	0	0	10	9.7	55	53.3	65	63.1
Total	994	20	2.01	19	1.9	67	6.7	203	20.3	289	29.0

Table VIII

Quantitative Analysis of Total Number Cases With Light, Medium, and Severe Periodontal Disease

Age in Years	No. Exam.	Normal		Slight		Medium		Severe		Total with Disease	
		No.	%	No.	%	No.	%	No.	%		
15-41	994	20	2.01	275	27.6	251	25.2	448	45.0	974	97.8

Table IX

Relationship of Age to Total Periodontal Disease, Total Calculus, and Number of Carious Teeth per Case

Age in Years	No. Exam.	Total Calculus		Total Periodontal Disease		No. Carious Teeth per Case*	
		No.	%	No.	%	No.	%
15-20	256	207	80.8	242	94.5	279	108.9
21-25	186	166	89.2	181	97.3	237	127.4
26-30	217	207	95.3	217	100.0	213	98.1
31-35	135	132	99.7	134	99.2	159	117.7
36-40	97	97	100.0	97	100.0	126	129.8
41-over	103	103	100.0	103	100.0	264	256.3
Total	994	912	96.6	974	97.9	1248	125.5

*No. carious teeth per case = simple comp'd cavities and teeth to be extracted because of caries.

DISCUSSION

Aside from the quantitative and qualitative analysis of this study, no serious attempt is made to theorize the etiology of caries, periodontal disease, or calculus. The literature amply discusses the numerous systemic, local, and exciting factors.

A study of the accumulated data showed that the caries index was much lower than that revealed in other countries with a higher standard of living.[1-3] On the other hand, the periodontal disease index was much higher than that of the United States or England.[6] Schlack[5] showed a 3.72 average for simple and compound cavities in 71,015 United States Naval personnel in sharp contrast to 0.95 average for the Egyptian study group. The regional difference of the incidence of dental defects, periodontal disease, and calculus formation, slightly higher in Lower Egypt, may in part be explained by the adoption of European foods in Lower Egypt.[4, 9] On the other hand, in accordance to what Mills[10] reported on rivers in the United States, there appeared to be in Egypt some increase in the incidence to caries as one passes from the headwaters to the mouth of a river. Mills' explanation is that this increase is due to soil leaching.

It is probably due to the lack of preventive dental treatment throughout Egypt that teeth to be extracted ran proportionally high, as 26.5 teeth were in need of extraction for every 100 patients. As expected, patients when classified according to age revealed that the incidence of dental defects, periodontal disease, and calculus deposits increased rapidly after the age of 30 years, the periodontal conditions becoming aggravated by the increase of dental defects and calculus, and the dental defects becoming more numerous because of the resulting periodontal conditions. The high incidence of periodontal disease (97.7 per cent) closely paralleled oral sepsis, amount of calculus, dental defects, and apparent nutritional deficiencies. The number of patients with severe (3-) periodontal condition was approximately twice that of the medium (2-) or slight (1+) cases. This numerical increase may be due partially to the inclusion of those cases with ulcerative gingivitis and periodontosis as severe. The incidence of gingivitis was high, and as age increased there was a steady trend of the disease to progress to the more chronic and destructive forms. As many of the patients manifested diets insufficiencies, that is, scurvy, rickets, and pellagra, it is reasonable to believe that the periodontal tissues were influenced by this chronic malnutritional state.

However, no appraisal of the nutritional intake would be indicative without consideration of the high incidence of intestinal parasites. A recent survey[11] revealed that 100 per cent of the fellaheen in a representative village were infected with one or more protozoa. The maximum number of species found in any one stool specimen was seven. It is of interest to note that 65 per cent had the most pathogenic protozoa, Endamoeba histolytica, the cause of amoebiasis, and after the ages of 2 to 5 there was no specific immunity to any one species of protozoa.

TABLE X

RELATIONSHIP OF AGE TO ABSENCE OF PERIODONTAL DISEASE, CALCULUS, AND CARIES

AGE IN YEARS	NO. EXAM.	NO CALCULUS		NO PERIODONTAL DISEASE		NO CARIES	
		NO.	%	NO.	%	NO.	%
15-20	256	49	19.1	14	5.4	31	12.1
21-25	186	20	10.7	5	2.6	12	6.4
26-30	217	10	4.6	0	0	10	4.6
31-35	135	3	2.2	1	.17	12	8.7
36-40	97	0	0	0	0	7	7.2
41-over	103	0	0	0	0	7	6.7
Total	994	82	8.1	20	2.0	79	7.9

TABLE XI

RELATIONSHIP OF AMOUNT OF CALCULUS TO AGE AND REGION OF BIRTH

AGE IN YEARS	NO. EXAM.	PERSONS WITH CALCULUS						PERSONS WITHOUT CALCULUS	
		HEAVY		LIGHT		NONE			
		NO.	%	NO.	%	NO.	%	NO.	%
Upper Egypt									
15-20	90	28	31.1	57	63.3	15	16.6	75	83.0
21-25	68	36	52.9	23	33.8	9	13.2	59	86.7
26-30	76	50	65.7	24	31.5	2	2.6	74	97.3
31-35	60	38	63.3	22	36.6	0	0	60	100.0
36-40	40	26	65.0	14	23.4	0	0	40	100.0
41-over	27	21	77.7	6	22.2	0	0	27	100.0
Total	361	199	55.1	146	40.4	26	7.2	335	92.7
Lower Egypt									
15-20	166	37	22.2	95	57.2	34	20.4	132	79.5
21-25	118	62	52.5	45	38.1	11	9.3	107	90.6
26-30	141	84	59.5	49	34.7	8	5.6	133	94.3
31-35	75	48	64.	24	32.	3	4.	72	96.
36-40	57	36	63.1	21	36.8	0	0	57	100.0
41-over	76	62	81.5	14	18.4	0	0	76	100.0
Total	633	329	51.9	248	39.1	56	8.8	577	91.1

TABLE XII

RELATIONSHIP OF TYPE OF CALCULUS TO AGE AND REGION OF BIRTH

AGE IN YEARS	NO. EXAM.	SALIVARY		SERUMAL		COMBINATION		NONE		PERSONS WITH CALCULUS	
		NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
Upper Egypt											
15-20	90	11	12.2	7	7.7	57	63.3	15	16.6	75	83.3
21-25	68	7	10.29	4	5.8	48	70.5	9	13.2	59	86.7
26-30	76	6	7.8	5	6.5	63	82.8	2	2.63	74	97.3
31-35	60	10	16.6	4	6.6	46	76.6	0	0	60	100.0
36-40	40	7	17.5	3	7.5	30	75.0	0	0	40	100.0
41-over	27	3	11.1	4	14.8	20	74.0	0	0	27	100.0
Total	361	44	12.1	27	7.4	264	73.1	26	7.2	335	92.7
Lower Egypt											
15-20	166	15	9.03	9	5.4	108	65.06	34	20.4	132	79.5
21-25	118	5	4.2	11	9.3	91	77.1	11	9.3	107	90.6
26-30	141	7	4.9	15	10.6	111	78.7	8	5.6	133	94.3
31-35	75	8	10.6	6	8.0	58	77.3	3	4.0	72	96.0
36-40	57	0	0	0	0	57	100.	0	0	57	100.0
41-over	76	0	0	0	0	72	91.0	0	0	76	100.0
Total	633	35	5.5	41	6.4	497	78.5	56	8.8	577	91.1

TABLE XIII
RELATIONSHIP OF TYPE OF CALCULUS TO AGE AND PERIODONTAL DISEASE

AGE IN YEARS	NO. EXAM.	SALIVARY		SERUMAL		COMBI-NATION		TOTAL NO. WITH CALCULUS		GINGIVITIS				ULCER-ATIVE		MARGINAL PERIO-DONTITIS		PERIO-DONTOSIS SYNDROME		TOTAL NO. WITH PERIODONTAL DISEASE	
										MARGINAL		HYPER-TROPHIC									
		NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
15-20	256	26	10.1	16	6.2	165	64.4	207	80.8	144	56.2	47	18.3	14	5.4	23	8.9	14	5.4	242	94.5
21-25	186	12	6.4	15	8.06	139	74.7	166	89.2	101	54.3	15	8.0	8	4.3	52	27.9	5	2.6	181	97.3
26-30	217	13	5.9	20	9.2	174	80.1	207	95.3	120	55.2	27	12.4	11	5.6	56	25.8	3	1.3	217	100.0
31-35	135	18	13.3	10	7.4	104	77.	132	99.7	46	34.6	9	6.6	7	5.1	60	44.4	12	8.8	134	99.2
36-40	97	7	7.2	3	2.2	87	89.6	97	100.0	29	29.8	14	14.4	4	4.1	33	33.6	17	17.5	97	100.0
41-over	103	3	2.9	4	3.8	92	89.3	103	100.0	15	14.5	12	11.6	6	5.8	65	63.1	5	4.8	103	100.0
Total	994	79	7.9	68	6.84	761	76.5	912	96.6	455	45.7	124	12.4	50	5.0	289	29.0	56	5.6	974	97.9

This observed difference in the indices of caries and periodontal disease may be answered in part when one considers the nutritive loss of solid foods being partially replaced by the fellaheen's large volume of water intake. As the large majority of villages are situated along the Nile River with its reported high mineral content (Table XIV), a readily assimilated food, vital to normal tooth formation, is accessible. On the other hand, the chronic intestinal infections, the chief cause of which is contaminated water, lowers the body's resistance and thus increases the susceptibility to oral and other body infections. The combination of oral sepsis and low tissue resistance in itself furnishes sufficient cause for periodontal disease.

In view of the much publicized protective influence of fluorines on developing teeth, the concentration of fluorides in Nile water at Cairo was tabulated as shown in Table XIV. Inasmuch as the yearly average concentration of 0.71 ppm indicates a threshold within normal, during the dry season months of May and June when the Nile is at its lowest level, the concentration of fluorides rises to 0.9 ppm. Mottled enamel is not uncommon among the Egyptian populace, but to date no statistical data have been accumulated with regard to its geographical distribution, although the consensus is that mottled enamel is more prevalent in the villages of Upper Egypt.

TABLE XIV

ANALYSIS OF NILE WATER AT CAIRO, EGYPT, 1946-1947*

YEARLY AVERAGE	MINERALS
30.5	Calcium
8.8	Magnesium
16.6	Sodium
NIL	Phosphates
.026	Iron
0.71	Fluorides

*Computed from the personal files of Dr. H. H. Rashid, Director of Water Section, Public Health Laboratories, Cairo, Egypt.

SUMMARY

1. Analysis of the dental records of 994 Egyptian fellaheen from Upper and Lower Egypt revealed 1.36 carious teeth per carious case.

2. The caries index was higher in Lower Egypt (1.1 average per person) than in Upper Egypt (0.81 average per person).

3. Periodontal disease varying from slight to severe was found to be present in 97.7 per cent of the study group.

4. Marginal gingivitis was the most common of the periodontal diseases found.

5. Periodontal disease bears a relationship to the amount and type of calciferous deposits, malnutrition, and dental defects.

6. Malnutrition is strongly influenced by the high incidence of intestinal parasites.

7. Geographical distribution of periodontal disease was not considered significant.

8. Dietary insufficiencies, induced by intestinal parasites and inadequate food intake, in addition to oral sepsis are perhaps the most important predisposing factors to periodontal disease in Egypt.

9. The yearly concentration of fluorides (0.71 ppm) in the water supply at Cairo, may be an indication that fluorine exerts a protective influence against tooth decay in Egypt.

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DETERMINATION OF SIZE AND SHAPE OF THE APATITE PARTICLES IN DIFFERENT DENTAL ENAMELS AND IN DENTIN BY THE X-RAY POWDER METHOD

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INTRODUCTION

IT IS a well-known fact that the apatite particles in dental enamel, dentin, and bone are of colloidal dimensions, that is, the particle dimensions are less than 0.2 μ or 2,000 Å.

Möller and Trömel[1] gave the figure 1 $\mu = 10^4$ Å for the particle size of enamel and 0.1 $\mu = 10^3$ Å for the particle size of dentin. Those figures were accepted and cited by Brandenberger and Schintz.[2] Yardeni[3] gave 0.3 μ = 3,000 Å as an average size, while Bale, Hodge, and Warren[4] gave 270 Å for enamel particles and 240 Å for dentin. Thewlis[5] stated, "The size of the crystalline particles in human enamel has been estimated by Bale and associates to be about 0.3 μ." This is undoubtedly due to an error in printing; the correct value is 0.03 μ.

A. Tovborg Jensen and Eva Möller[6] pointed out that the particle size in an enamel layer may decrease with increasing distance from the surface of the tooth. This effect could be seen, though less clearly in the diagrams published by Trömel and Möller, but they did not comment on it.

The particle size and its variation are of considerable odontological interest and should be taken into account whenever differences in enamel hardness are explained and when quantitative analyses of tooth and bone substance are discussed. The particles are so small that a monomolecular surface layer amounts to several per cent of the bulk material. Because of this fact, deductions of formulas (hydroxyl-apatite versus "carbonate apatite," the role of water, magnesium, and alkali metals in dental enamel, etc.) should be made with great care.

The minute particle size of enamel, dentin, and bone, until now, have been observed only from the broadening of lines in x-ray powder diagrams, and our knowledge of the particle size is of an approximate character.

Trömel's very large figure (see the following) seems to be due to an error in calculation. Particles of 1,000 Å or 10,000 Å should give no perceptible broadening in small cameras (57 mm. diameter) such as used by Trømel.

The figures given by Bale, and associates[4] are much more reliable, but even so, the experiments seemed worth repeating on different kinds of enamel with a more refined technic. Furthermore, the shape of colloidal apatite particles apparently have not been discussed before.

Bale and co-workers[4] gave only one figure, but, as pointed out earlier, the particle size is not a single figure. The x-ray powder method should first

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provide some knowledge of the *range* within which the particle sizes of enamel may be expected and then we should look for correlations between particle size and properties of different dental enamels. As pointed out in an earlier publication,[6] better defined values might have been obtained by using a back-reflection technic,[7] but lack of suitable apparatus has prevented us from applying this method.

EXPERIMENTAL TECHNIC AND CALCULATIONS

The experiments were carried out with three specimens of human enamel: incisor, premolar, and upper third molar, one of human dentin, one of rabbit enamel, and one of beaver enamel. (The latter was interesting because of the pigmentation due to a content of ferric ions.)*

The intensity-distribution curves were recorded by an automatic Zeiss-microphotometer with slit-width of 0.17 to 0.41 mm., depending on the line-breadth, and slit-length of 0.71 mm.

The x-ray diagrams were all taken with a circular camera having a diameter of 191 mm., a rectangular slit, reference knives according to Phragmén-Bradley, and calibrated with NaCl. Co-K_α -radiation was employed. ($\lambda\alpha_2$ = 1.78891 Å and $\lambda\alpha_2$ = 1.79282 Å).

We will not discuss here the many methods suggested for measuring line-broadening and for converting line-broadening into sizes and shapes of the particles (Scherrer,[8] Warren and Biscoe,[9] Taylor,[10] A. Tovborg Jensen,[11] F. W. Jones,[12] and Kochendörfer.[13]) We shall describe our method and try to give sufficient data to permit, if necessary, the recalculation of particle size and shape according to some other method described in the literature.

The intensity distribution in an x-ray powder line is given as a curve as shown in Fig. 1. In determining the particle size and shape from such curves, we proceeded as follows:

1. *Determination of the "half-peak width"* (that is, the angular range [in radians] between two points where the intensity has fallen to one-half its maximum value).

(a) of a line (hkl) from the diagram of a well-crystallized powder (average crystallite size $> 10^{-4}$ cm.) and

(b) of the same line (hkl) from a specimen of the same material but of colloidal particle size.

The two diagrams are taken with the same x-ray camera in exactly the same position and in order to exclude errors due to differences in absorption, the two powder rods are of the same thickness.

The measured values are called B_m and B_m* in the following:

2. *Corrections of the measured B_m values for broadening due to finite photometer slit-width and imperfect resolution of the α_1 α_2 doublets.* The reduced values are called $B_{m(red)}$ and $B^*_{m(red)}$.

*We are very much indebted to J. J. Pindborg for procuring and preparing the samples, and we wish to thank him for valuable discussions of odontological and histological problems.

3. *Graphical adjustment of the reduced line breadths for the well-crystallized powder.* The smoothened curve gives the "average line breadth" at any value of the glancing angle. This figure is called $B^{**}_{m(red)}$.

4. *Calculation of the pure diffraction broadening* b_{hkl} *given by:*

$$b_{hkl} = \sqrt{(B^{*}_{m(red)})^2 - (B^{**}_{m(red)})^2}$$

5. *Calculation of the "apparent crystallite size"* ε *for any reflection* given by

$$b_{hkl} = \frac{\lambda}{\varepsilon \cos\vartheta}$$

where λ is the wave length and ϑ the glancing angle.

6. *Discussion of the crystallite shape indicated by the values of* ε.

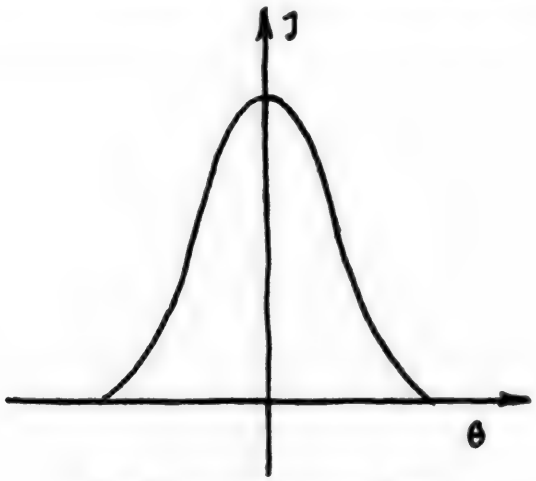

Fig. 1.—Intensity distribution curve.

The six steps of the procedure are explained in the following discussion:

1. *Determination of the "Half-peak Width":*

In order to determine the line breadth we have registered on the same photometer record a part of the x-ray diagram (from 0002 to 0004) (Fig. 2, curve *R*) and a calibration strip (curve *C*).

The strip was printed by exposing the film to the primary beam through a slit before which a wheel, shaped like an Archimedean spiral, was rotating. In this way we obtained a strip in which the amount of radiation received at any point is proportional to the distance from the end of the strip. Hence, the curve *C* depicts the photometer inflection as a function of the amount of the radiation received at the corresponding point on the film.

The top and the ground of a peak are projected on the calibration curve *C* in *A* and *B*. *AB* is projected on a horizontal line in *A′ B′*. The middle point of *A′ B′* is related to the calibration curve in *M*. In this height the "half-peak width" is measured.

2. *Corrections:*

a) Photometer Slit-width:

If the photometer slit-width is called p; the measured line breadth, B; the reduced line breadth, $B_{(red)}$, then we have according to Kochendørfer (p. 448):

$$B_{(red)} = B\left[\tfrac{1}{2}\ (1 + \sqrt{1 - \frac{p}{B}}\ )\right] \text{ when } \frac{p}{B} < 1.$$

b) α_1 α_2—doublets:

According to Kochendörfer (p. 449):

$$B'_{(red)} = B_{(red)}\left[\ \tfrac{1}{2}\ (1+ \sqrt{1 - \frac{s}{B_{(red)}}})\right] \text{ where } s = 4/3.2R\ \frac{\lambda\alpha_1 - \lambda\alpha_2}{\lambda\alpha}$$

$tg\vartheta$, R is the camera radius, λ, the wave length of the radiation used.

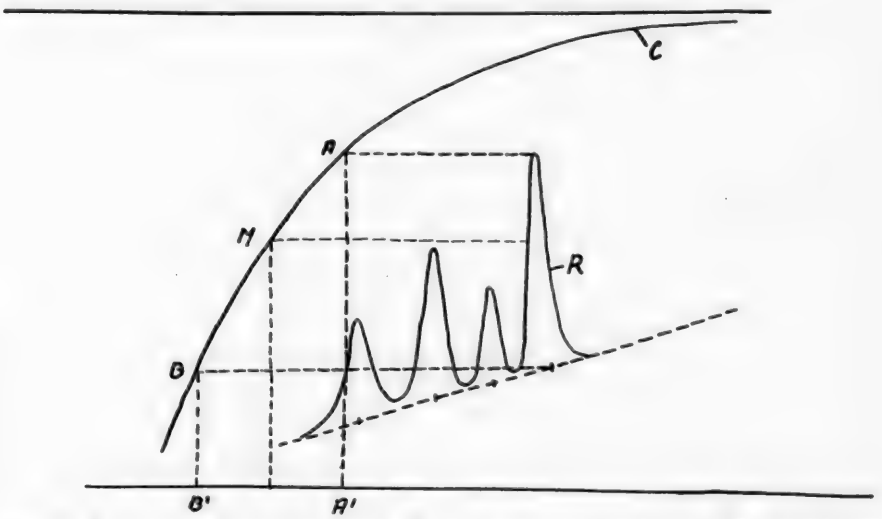

Fig. 2.—Intensity distribution curve (*R*) and calibration curve (*C*). The stippled lines show how the point where the intensity has fallen to one-half its maximum value is determined.

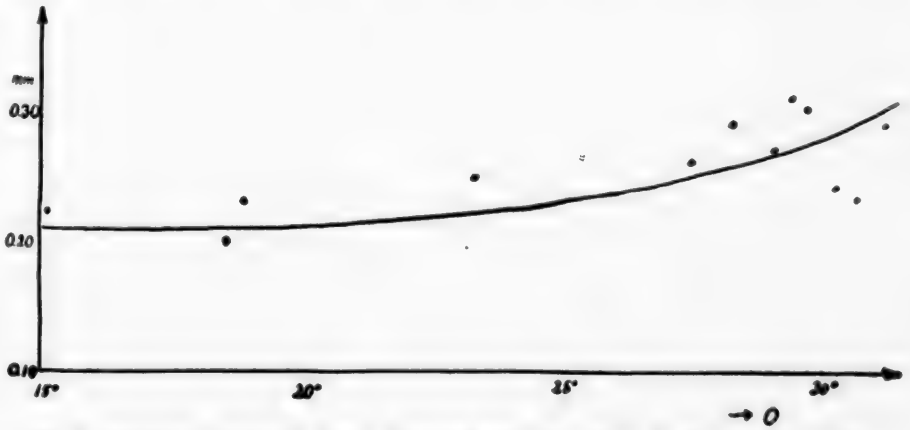

Fig. 3.—Graphical adjustment of the reduced line-breadth values for the mineral apatite.

3. *Graphical Adjustment:*

The reduced line-breadth values for the well-crystallized powder (a beautiful, transparent apatite from the sedimentary rock Alnø-lime Sweden) are plotted as a function of the glancing angle, and a slightly ascending curve is drawn (Fig. 3).

The points are seen to be evenly distributed around the curve independent of the indices. Hence this curve gives the variation of the line breadth with the glancing angle for the well-crystallized powder and we have employed B^{**}_{red} read from the curve rather than individual measurements in our subsequent calculations of the diffraction broadening.

Points 4 and 5 need no further explanation.

6. *The Crystallite Shape Indicated by the Different Values of ε:*

According to Laue[14] and Brill,[15] it is possible to deduce a probable crystallite shape from a series of ε -values.

Supposing that the particles belonging to the hexagonal system are of parallelepiped shape, ε is given by (Fricke and Lücke[16]):

MEASURED AND REDUCED LINE-BREADTH VALUES, VALUES OF THE PURE DIFFRACTION BROADENING AND T...

NO.	SPECIMEN: PHOTOMETER SLIT-WIDTH — INDICES	GLANCING ANGLE	MINERAL APATITE: ρ = 0.170 MM. — B_m mm	$B^{**}_{m(red)}$ mm	HUMAN ENAMEL INCISOR: ρ = 0.345 MM. — B^{*}_{m} mm	$B^{*}_{m(red)}$ mm	b_{hkl} mm	ε	ε^1	HUMAN ENAMEL PREMOLAR: ρ = 0.345 MM. — B^{*}_{m} mm	$B^{*}_{m(red)}$ mm	b_{hkl} mm	ε	ε^1
1	0002	15.1°	0.31	0.21	0.51	0.36	0.29	600Å	600Å	0.75	0.61	0.57	310Å	3...
2	21$\bar{3}$1	18.5°	0.31	0.21	0.58	0.42	0.36	500–	470–	–	–	–	–	–
3	11$\bar{2}$2	18.8°	0.34	0.21	–	–	–	–	–	–	–	–	–	–
4	30$\bar{3}$0	19.1°	0.33	0.21	0.58	0.42	0.36	500–	480–	–	–	–	–	–
5	20$\bar{2}$2	19.9°	0.30	0.21	0.54	0.38	0.32	570–	540–	1.00	0.85	0.82	220–	2...
6	31$\bar{4}$0	23.3°	0.38	0.22	0.65	0.48	0.43	420–	450–	0.90	0.74	0.71	260–	2...
7	31$\bar{4}$1	24.7°	–	–	–	–	–	–	–	–	–	–	–	–
8	11$\bar{2}$3	25.7°	–	–	–	–	–	–	–	–	–	–	–	–
9	20$\bar{2}$3	26.6°	–	–	–	–	–	–	–	–	–	–	–	–
10	22$\bar{4}$2	27.5°	0.41	0.25	0.65	0.46	0.39	480–	480–	1.00	0.83	0.79	240–	2...
11	31$\bar{4}$2	28.3°	0.44	0.26	0.83	0.65	0.60	320–	470–	–	–	–	–	–
12	21$\bar{3}$3	29.1°	0.43	0.26	0.74	0.55	0.48	400–	520–	1.03	0.85	0.81	240–	2...
13	32$\bar{5}$1	29.8°	0.46	0.27	0.76	0.57	0.50	390–	440–	–	–	–	–	–
14	41$\bar{5}$0	30.3°	0.41	0.27	0.85	0.66	0.60	330–	460–	–	–	–	–	–
15	40$\bar{4}$2	30.7°	0.41	0.28	0.88	0.69	0.63	320	440	–	–	–	–	–
16	0004	31.3°	0.46	0.28	0.81	0.62	0.55	370–	600–	1.10	0.94	0.90	210–	3...

The empty places in the table indicate that we have been unable to determine the breadths of t...

$$\varepsilon = \sqrt{\frac{\frac{4}{3a^2}(h^2 + k^2 + hk) + (\frac{1}{c})^2}{(\frac{4}{3m_1, a_2})^2 [(h + k)^2 + \frac{1}{4}(h^2 + k^2)] + (\frac{1}{m_2c^2})^2}}$$

where hkl are the Miller indices and a and c the lattice constants. m_1 and ma_2 are pure numbers; the lengths of the edges of the crystal are then m_1a, m_1a and m_2c. From two different values of the ε m_1 and m_2 may be calculated.

Now it seems rather unreasonable to suppose a parallelepiped shape of an apatite particle. As mentioned before, apatite belongs to the hexagonal system, space group $C6_3/m$; that is, it has a sixfold screw-axis. Therefore, a six-edged prism is much more likely, but we do not expect to get more than a rough picture of the size relations.

RESULTS

The results are given in Tables I and II. In order to get comparable figures, all the m_1 and m_2 values are calculated from ε_{0002} and ε_{2242}. 0002 and 2242 are chosen, because they are well developed in all diagrams. ε^1 indicates the calculated values.

Apparently Crystallite Size'' Calculated for Human Enamel and Dentin, Rabbit Enamel, and Enamel

HUMAN ENAMEL THIRD UPPER MOLAR					RABBIT ENAMEL					BEAVER ENAMEL					HUMAN DENTIN				
ρ = 0.293 mm.					ρ = 0.414 mm.					ρ = 0.414 mm.					ρ = 0.517 mm.				
	$B_{m\,mm}$	$B_{m(red)\,mm}$	$b_{hkl\,mm}$	ε	ε^1	$B_{m\,mm}$	$B_{m(red)\,mm}$	$b_{hkl\,mm}$	ε	ε^1	$B_{m\,mm}$	$B_{m(red)\,mm}$	$b_{hkl\,mm}$	ε	ε^1	$B_{m\,mm}$	$B_{m(red)\,mm}$	$b_{hkl\,mm}$	ε	ε^1
69	0.57	0.54	330Å	330Å	1.01	0.85	0.82	210Å	210Å	0.85	0.69	0.66	270Å	270Å	1.08	0.88	0.86	200Å	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
79	0.66	0.63	290–	270–	–	–	–	–	–	0.75	0.60	0.56	320–	250	–	–	–	–	–
85	0.71	0.67	270–	210–	1.62	1.45	1.43	130–	160–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
00	0.84	0.80	240–	240–	1.39	1.20	1.17	165–	165–	1.09	0.90	0.87	220–	220	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
04	0.87	0.83	240–	260–	–	–	–	–	–	1.01	0.82	0.78	250–	260	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
35	0.97	0.93	220–	330–	–	–	–	–	–	1.27	1.07	1.04	190	270–	–	–	–	–	–

rticular lines.

TABLE II
M_1 AND M_2 VALUES AND THE CRYSTAL THICKNESS

	M_1	M_2	LENGTH IN C-AXIS DIRECTION M_2.C	LENGTH IN A AND B-AXIS DIRECTION M_1.A
Human enamel, incisor	66	87	600Å	620Å
Human enamel, premolar	33	45	310–	310–
Human enamel, third upper molar	30	48	330–	280–
Rabbit enamel	23	30	210–	220–
Beaver enamel	31	39	270–	290–

DISCUSSION

It is seen from the tables that the apatite crystallites in different dental enamels are of roughly spherical shape. This contrasts with what is found on apatite particles from bone where the particles are definitely elongated in the c-axis direction.* In one case of the enamel (third upper molar), there is perhaps an elongation in the same direction. This fact may be of importance because the c-axis is also the "fiber axis" both in enamel and in bone.

Furthermore, there is a significant difference in the quality of diagrams from different enamels. The incisor gave a beautiful diagram very like that from a hydroxyl apatite precipitated in alkaline solution by 100° Centigrade. The other enamels gave less fine diagrams.

We find that diagrams decrease in contrast with decreasing particle size in the specimen. The abatement in quality is very conspicuous, so it seems questionable whether this is due only to decrease in particle size. It may also be caused by a greater content of nonerystalline material in the enamels investigated.

In order to throw light on this problem, we intend to continue the investigations on material which has also been examined by crystal microscopical methods. Measurements of the absolute intensities of characteristic reflections will show whether the amount of amorphous substance is larger in those enamel specimens, which gave the poorer diagrams.

There is a "systematic error" in the measured dimensions: For low values of the glancing angle the calculated ε^1-values agree with the measured ones, but for higher ϑ-values the observed ε-values fall below the values calculated from ε_{0002} and ε_{2242}.

We have not yet been able to explain this fact satisfactorily. It may perhaps be due to polydispersity of the powder, to lattice-imperfections or to differences in lattice-constants for different particles. The latter alternative does not appear very likely. Furthermore, we do not know whether or not the undoubted orientation of the particles in the minute crystal aggregates of the powder may have some influence on the line-breadths.[5]

SUMMARY

Particle dimensions in enamel and dentin have not been determined with great accuracy up to now. We have determined the apparent particle size in

*Unpublished results by the authors.

a few enamel powders and in one dentin specimen and found that there are great differences in particle size in different enamels. Crystallite thickness ranging from some 600 Å in a fine human incisor to some 250 to 300 Å in human molars has been found. Figures for enamel from rabbit and beaver were somewhat lower.

The importance of particle sizes for discussions of enamel formulas is pointed out.

Enamel particles are uniformly developed in all directions; this contrasts with the apatite particles in bone which are elongated in the c-axis direction.

We have noted a curious effect in our enamel diagrams: The line broadening increases more with increasing particle size than expected for an assembly of error-free crystallites of uniform size. In other words, the apparent crystallite size calculated from higher order reflections is lower than that calculated from low-order reflections. This effect has not been explained.

The investigations are being continued.

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DENTAL CALCULUS

A Bacteriological and Physical Study

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INTRODUCTION

IT IS well known that areas of teeth covered with calculus are caries free and that calculus is considered as an indicator of inactive caries. Moreover, the calculus-covered lingual surfaces of the lower anterior teeth are caries resistant, even in a mouth with marked caries. Although cognizance of this fact has been taken for a good many years, suprisingly enough, no attempt was made at a closer approach to the study of the relation of calculus to caries resistance. Related to this question is the reflection of Box, "It appears that, from the caries standpoint, too much emphasis has been placed on periodic cleansing and polishing of the enamel surfaces and too little attention paid to their protection."[1]

It is a striking fact, indeed, that upon the removal of calculus, we invariably disclose a sparkling, smooth surace which imparts the unmistakable impression of high resistance to decay, though calculus includes among its organic constituents large numbers of bacteria, some of which are associated with caries[1] and with the formation of the deposit.[2] According to Naeslund,[2] the organic framework of calculus is provided by desquamated epithelial cells and certain filamentous microorganisms of the genus Leptotrichia. Nevertheless, a periodontal pocket, no matter how unhygienically it has been kept and no matter how much calculus has accumulated, is usually caries immune. But once the gingiva has receded and the calculus has been removed, the same area becomes caries susceptible. Cases of interproximal gingivitis and caries due to food impaction do not belong in this category.

What, then, is the nature of this protective factor which inactivates caries in areas covered by calculus?

At first thought, the conventional answer would be that the tooth structure, within the pocket is protected by the dental cuticle, and the detachment of the cuticle, through the removal of calculus or through abrasion, may predispose to caries. It is evident, however, that this phenomenon is more persistent and that the kind of resistance observed is of a quite unusual degree.

Alternatively, it is stated that the caries-preventive power of calculus may be due to its mineral content, which makes calcium available for the prevention of its withdrawal from the teeth.[3] Though this sounds plausible, it is, in fact, related more to the calcium content of saliva. Calculus is remarkably insoluble.[4] It does not diminish or disappear spontaneously; on the contrary, its hardness increases with time. In reality, it is steadily accumulating, without showing perceptible changes in constitution, surface texture, or degree of porosity.

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Our tests have shown that the permeability of calculus is relative. It is absolutely impermeable to gentian violet whereas it is stained through with a faint bluish color by the smaller molecules of methylene blue. With regard to the possibility of acid diffusion through calculus or to the neutralizing properties of calculus, our experiments undertaken to test the influence of calculus on the solubility of tooth structure in acid buffers and in saliva-candy mixture, proved negative. On the other hand, the remarkable absence of viable proteolytic bacteria in calculus, as found in our bacteriological tests, may be of importance. Though proteolytic bacteria of the filamentous type are enmeshed in the calculus, their role seems to be confined to the initial stages of precipitation. They are prominent in providing a tenacious framework for the deposition of calcium salts, or appear as active agents of calculus formation through their phosphatase enzyme.[5, 6]

The purpose of the present investigation was to determine bacteriologically whether some protective effect, some kind of immunizing principle against caries, could not be attributable to calculus as an entity, to its biosis and not to its mineral content.

Calculus is not formed at a single time. It accumulates gradually as it precipitates from the saliva layer by layer, adsorbing substances occurring in saliva and in blood.[1, 7] The mechanism of precipitation is a problem of colloidal chemistry, of the transition from an unstable, supersaturated solution into a stable, two-phase state.

Saliva releases not only minerals, but also mucin and whatever antibiotics there may be in saliva. These substances determining natural resistance, may infiltrate the various layers of calculus in the process of its formation. Mucin itself is not an inert substance for the growth of bacteria, but saliva contains also lysozyme, rhodan-kalium, antagonistic products of bacterial metabolism, and other still undefined inhibiting substances. Calculus, in general, may reflect the quality and activity of saliva, but calculus is solid and concentrated and is a by-product of daily activity. It can be more readily studied than saliva since it is stable, and, once formed, is less subject to variation and fluctuations.

BACTERIOLOGICAL STUDIES

Direct Smears.—Calculi freshly removed from the teeth were repeatedly washed in distilled water, dried on blotting paper, cut into internal and external portions, separately crushed on slides, and the smears stained with Gram's method.

Findings.—These smears showed, among the calculus debris and epithelial cells, great numbers of gram-positive threads, some of them morphologically identified as leptotrichia,*[18] forming a tangle with chains of gram-positive threads. In individual cases numerous Borrelia were present. Gram-negative cocci were scarce. The internal surface of the calculus showed a much poorer flora than the external part and was almost sterile.

Ed. Note: According to Bergey's *Manual of Determinative Bacteriology*, 6th edition (1948), the so-called leptotrichia are apparently better placed in other genera (pp. 364-365).

Cultural tests.—

1. *Solid Media:* Calculus was obtained from twenty-three patients, and 108 calculi flakes were tested. Calculi freshly removed from the teeth, coated with saliva and whatever adherents were present, were placed on agar with 3 to 5 per cent tomato juice[8] or on blood agar.

Efforts were made to release the subgingival calculus without injuring the gingival margin in order to avoid bleeding. This succeeded on most occasions. Cases were taken as they came at random. The flakes of calculus were dropped upon the agar from the sterile cleaning instrument with as few manipulations as possible. After forty-eight hours of incubation at 37° C., the growth immediately surrounding the calculus was examined.

(*a*) *Calculus:* Macroscopically quite a distinct and typical appearance was observed. The flakes of calculus were surrounded by confluent growths 1 to 3 mm. in diameter (Fig. 1), predominantly gram-negative. It consisted of gram-negative cocci (in most cases morphologically identical with Neisseriae), a number of different types of gram-negative rods and filaments and occasional

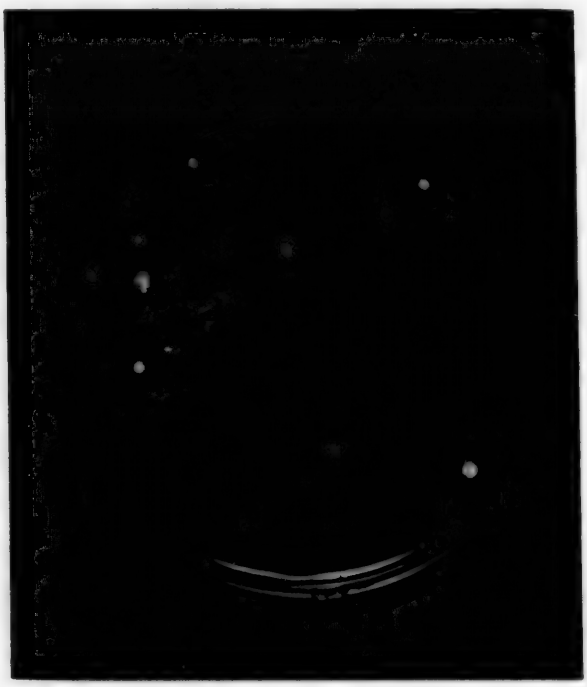

Fig. 1.—Flakes of calculus planted on agar with tomato juice. The continuous line presents a saliva streak. The flakes of calculus are surrounded by a confluent growth, predominantly gram-negative. The two crossed streaks of saliva from the same patient show a proliferation of cocci and lactobacilli, gram-positive in character.

streptococci, lactobacilli, gram-positive threads, diphtheroids, fusiforms, and borrelia. Some flakes showed no growth at all; a few others, gram-negative rods only.

Results obtained with blood agar were identical.

(*b*) *Saliva:* In a parallel series of studies, loopsful of saliva were separately streaked. Upon incubation at 37° C. for forty-eight hours they showed a predominantly gram-positive flora (lactobacilli and streptococci).

2. *Fluid Media.*—A piece of calculus and a loopful of saliva were separately sown into nutrient broth plus 3 per cent neutral tomato juice. After forty-eight and ninety-sixth hours of incubation at 37° C., diffuse and homogenous growth in test tubes with calculus was observed whereas in those with saliva the growth appeared as a granular deposit with little turbidity in the supernatant fluid. The calculus was found to be covered with a soft curdle which microscopically showed a mixture of gram-negative cocci and gram-negative rods with occasional gram-positive organisms (as above). The flora in the media with saliva was predominantly gram positive.

Identification.—The gram-negative rods from the various plates were subcultured by continuous replating, and their biological and physiological properties were established with the aid of routine biochemical tests. Four different types were distinguished, all nonsporogenous, producing indol, and Voges-Proskauer negative:

Type I consisted of nonmotile short rods, with bipolar staining, almost resembling cocci. These coccoid rods grew well also under anaerobic conditions. They did not attack glucose, lactose, or sucrose and grew well on citrate-agar, with production of acid.

Type II presented very minute motile rods, staining weakly with Gram's counterstain. Colonies were slightly mucoid and lobate. Glucose, lactose, sucrose, methyl-glucoside, and cellobiose were not attacked. Slow utilization of citrate and slow liquefaction of gelatin were noted. (Type II is weakly proteolytic.)

Types III and IV, of the coli-aerogenes group, were methyl-red positive, Voges-Proskauer negative, and appeared to belong to the genus Citrobacter[9] or to one of the types described by Tittsler and Sandholzer.[10] Type IV differed from Type III in ability to ferment sucrose with production of acid and gas. In several instances, the growth around the calculus was found to be a pure culture of Type IV. No attempts at identification of the gram-negative cocci were made.

DISCUSSION

This is a report on a series of bacteriological tests undertaken to ascertain in what manner calculus exerts its protective properties against caries. Though it is common clinical experience that a tooth area covered with calculus is caries free, this knowledge remained empirical. The idea that cal-

culus, contaminated as it is, should be examined for its antibacterial properties, may sound paradoxical to anyone familiar with oral bacteriology. The findings, nevertheless, seem to be significant in that they may point to a new approach to the study of caries immunity.

Calculus, unlike tooth structure, is not subject to decay, since proteolysis or fermentation has but little effect on it. Its caries-preventive principle, not affected even by the presence in the pocket of pus and products of tissue autolysis, may be derived from some factors favorable to proliferation of a predominant gram-negative flora, or may be traced to the summary effect of inhibitory substances present in saliva and blood. Local alkalinity may play a part in this phenomenon.[15]

Our direct smears of crushed calculus showed the same predominancy of gram-positive filaments described by Bibby.[11] Stained smears from the undersurface of crushed calculus showed almost no microorganisms. This is concordant with the findings reported by Taylor.[12]

However, Bibby's conclusion that "there were definitely more gram-positive filaments in calculus than elsewhere in the mouth" seems to be incomplete, in view of our findings. It is correct with regard to the microorganisms enmeshed in the calculus itself. But the immediate surroundings of the calculus, as well as the viable forms in culture media, are predominantly gram negative. According to our investigations, three distinct zones may be pictured with regard to the predominant microorganisms: (1) The calculus itself, with its petrified gram-positive filaments of the actinomyces type; (2) the zone encircling or adjacent to the calculus with gram-negative cocci and rods (in some specimens a pure culture of rods only, of the Type IV); and (3) the oral flora, in general, with the predominance of gram-positive cocci and bacilli.

In a few tests of plates inoculated with both saliva and calculus, a clear-cut distinction in growth between the plate as a whole and the zone of calculus can be demonstrated (Fig. 1). On the one hand, lactobacilli, yeast, cocci, and leptotrichia proliferated, all gram-positive; on the other hand in smears from areas adjacent to the caculus, gram-negative bacilli predominated. In the thinner streaks of saliva, the lactobacilli, which multiplied in tiny beaded colonies on the lines of inoculation, thinned out and vanished (phenomenon of limitation of stationary population) whenever they encountered a piece of calculus. Their growth was interfered with by its presence. In heavy streaks, we missed this bacteriostatic phenomenon.

Subgingival calculus includes both products of salivary secretion and exudative products from the gingivae. The question arises as to whether calculus, by virture of these substances, is antagonistic to the growth of at least certain gram-positive organisms.

With respect to caries inhibition, it seems pertinent to mention the work of Picard[13] who studied the behavior toward Gram's stain exhibited respec-

tively by bacteria of the healthy and the diseased mouth. According to his findings, in diseased mouths gram-positive bacteria predominate, and in healthy mouths, gram-negative.

We should like to stress one more point, namely, that almost no proteolytic bacteria no proteus, could be cultivated from the calculus. This is the more surprising, since the oral cavity has a natural supply of these organisms, and since the nutritional requirements of the proteolytic bacteria were entirely satisfied by the media used.

The fact that leptotrichia are intimately associated with calculus formation does not refute our findings. We have already mentioned that these seem to be important in the initial stages only of calculus formation; in fact, calculus has been defined as a petrified mass of leptotrichia.[14] Calculus is odorless, and the initial layer of calculus, the innermost deposit, is almost void of microorganisms.

SUMMARY AND CONCLUSIONS

1. Calculus is correlated with caries resistance.

2. Calculus occupies a critical bacteriological line, on the boundary between caries and periodontal disease.

3. In the zone adjacent to calculus, gram-negative bacteria, predominantly, are capable of establishing themselves.

4. Proteolytic bacteria are almost absent in the zone of calculus.

5. Calculus seems to possess some weak antibiotic properties.

6. Calculus biosis should share priority in attention, together with the major problems of caries research.

7. Any suggestion that our findings may be regarded as prejudicial to the removal of calculus is erroneous.

THE INHIBITORY INFLUENCE OF CALCULUS ON THE GERMINATION OF WHEAT GRAINS

Method.—The experiment was conducted in pairs of Petri dishes, with filter paper fitted on the bottom of each plate. Seven or eight cubic centimeters of distilled water were poured into each plate, and fifty grains of wheat seeded. Into one of each pair of dishes several flakes of calculus were added. The second one was kept for control. The calculus was freshly removed or taken from a stock where it was kept dry for a few days. The calculus was thoroughly washed in distilled water prior to its introduction into the Petri dish. This experiment was repeated five times with calculus derived from different patients or mixed from two or three mouths. The dishes were kept at room temperature or in a thermostat at 25° centigrade.

The progress of germination was studied from observations made on the rate of germination, the final percentage of germinated seeds, and the length of germinated radicles.

CONCLUSIONS

1. In all five experiments the wheat germs were affected by the presence of calculus in the Petri dish, as compared with the control dish (Figs. 2 and 3).

2. Calculus inhibits the germination, as evidenced both by the smaller percentage of germinated grains and by the shorter radicles. The inhibition of the coleoptile growth is inappreciable.

3. The degree of inhibition varied from 50 to 8 per cent. However, we were unable to determine, from the small number of experiments whether the degree of inhibition varies with such factors as age, caries, resistance, type of calculus, and so forth.

4. There is no difference in the final inhibition rate between germination at room temperature and with a thermostatic control. As a general phenomenon, the germination at room temperature is retarded, but the influence of calculus is not affected.

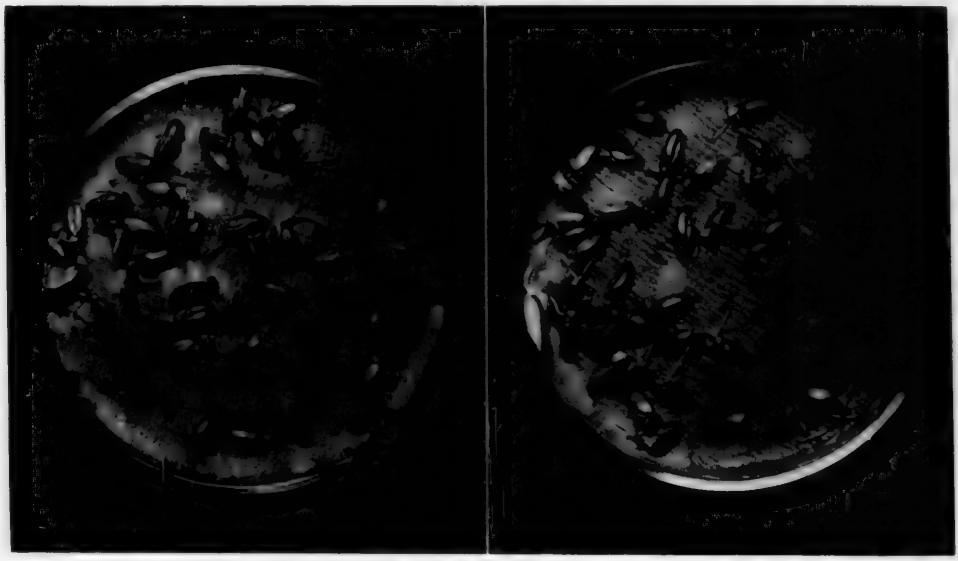

Fig. 2.—Petri dishes with calculus showing inhibition of germination.
Fig. 3.—Germination of wheat. Control dish. Experiment No. 5.

DETERMINATION OF THE PRESENCE OF CALCIUM CARBONATE

A polished microsection of calculus was stained in a molar solution of copper nitrate. Calcium carbonate, if present, forms a soluble calcium nitrate and following the immersion in concentrated ammonia for a few seconds, a cupric complex is produced which imparts to the section a dark blue color. No change in color, however, resulted in our case after the specimen had been treated in this way. It seems that there was no calcium carbonate present in the calculus section.

TABLE I

EFFECT OF CALCULUS ON GERMINATION OF WHEAT

NO.	DATE	GERMINATION PERCENTAGE		LENGTH OF RADICLES (MM.)		LENGTH OF COLEOPTILE		TEMPERATURE
		CALCULUS	WATER	CALCULUS	WATER	CALCULUS	WATER	
I	1/7/47	53	97	5.0	10.0	–	–	Room
	1/10/47	95	100	22.0	42.0	5.0	12.5	
II	1/14/47							Thermostat 25° C.
	1/16/47	74	92	27.0	42.0	10.0	10.0	
III	1/23/47							Thermostat 25° C.
	1/24/47	2	18	3.0	8.0	1.0	3.0	
	1/26/47	14	100	30.0	82.0	27.0	38.0	
IV	1/28/47							Thermostat 25° C.
	1/30/47	64	100	35.0	45.0	11.0	11.0	
V	1/16/47							Room
	1/21/47	80	92	8.0	15.0	2.0	6.0	

POLAROSCOPIC EXAMINATION

A thin section of calculus mounted in Canada-balsam was examined in polarized light with crossed Nicols. The examination revealed that the structure in general is concentrically laminated. Alternative dark and light layers follow in sequence, with spaces between them (Fig. 4).

The dark layer has a wavy outline. Its appearance is reminiscent of a cumulus. On rotation between crossed Nicols it shows no change in color. It seems, therefore, to be amorphous or crypto-crystalline in pattern. The light layer has a fibrous texture, the fibers being arranged more or less perpendicu-

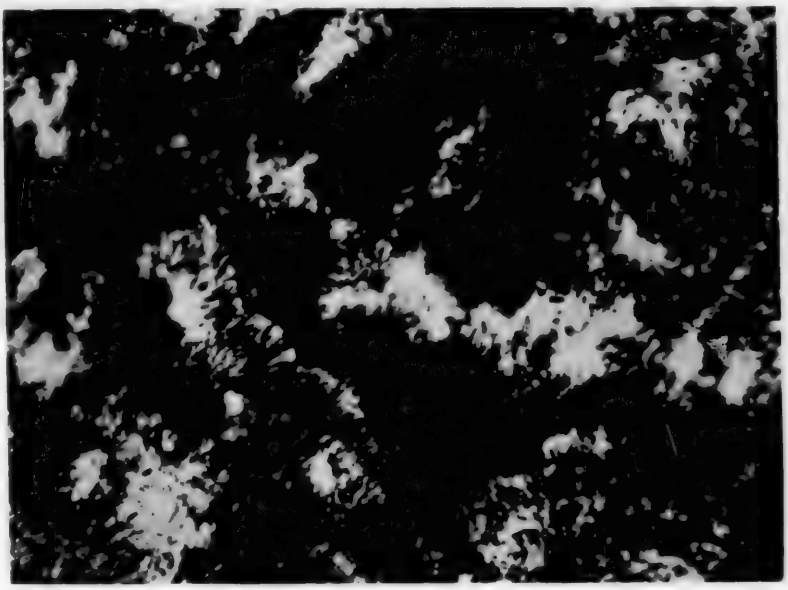

Fig. 4.—Microsection of calculus. Concentrically laminated structure. Alternative light and dark areas.

larly to the dark layers. Sometimes they seem to radiate, appearing as a rosette. With Crossed Nicols, the fibers react by direct extinction; when hooked with the inserted gypsum plate, they change the color from yellow to blue.

On this polarasocopic evidence of direct extinction and color change, it may be inferred that the structural unit of dental calculus is apatite. It possesses the properties of low birefringence and of preferential orientation. The amorphous appearance of the dark layers may be due to the organic reticulum of the calcareous crust.

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SALIVARY CITRATE CONTENT AND EROSION OF THE TEETH

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McCLURE and Ruzicka[6] observed the destructive action of citrate ions on rat tooth surfaces. Their experimental animals ingested large amounts of citrate in drinking water adjusted to a pH of 5.5 to 7.2 and areas on the lingual of the molars were decalcified. The possibility that this process might be related to erosion was suggested to the authors by the resemblance between the lesions illustrated by McClure and Ruzicka[6] and the usual clinical appearance of erosion.

It appeared that studies of the citrate content of the saliva of individuals with and without erosion should be of value. Earlier studies of the citrate content of saliva showed variations from zero to 1.3 mg. per 100 cubic centimeters. Kuyper and Mattill[5] stated, "Apparently, saliva is the only body tissue tested in which citrate has not been detected." Pucher, Sherman, and Vickery,[9] however, found doubtful quantities of citrate in saliva from three individuals and 0.4 to 0.33 mg. per 100 c.c. in four others. From one individual 1.3 mg. per 100 c.c. of saliva was obtained three hours after grapefruit had been eaten, although the saliva of this subject had shown only traces previously. Dickens,[2] in a report on citric acid content of tumor and bone tissue, cited Östberg,[7] Schersten,[11] and Grönvall[4] as reporting that cerebrospinal fluid, amniotic and follicular fluids, blood serum, aqueous humor, saliva, and sweat contain diminishing quantities of citrate from 5 to 0.1 mg. per 100 cubic centimeters.*

The first problem was to select a suitable analytic method for salivary citrate, then to establish "normals," and finally, to compare the salivary citrate quantity of individuals with erosion and without erosion.

METHOD

Clinical:

To obtain sufficient patients with erosion, the teeth of 1,345 male freshman students at Ohio State University were examined at the time they received their entering physical examination. In this group, twenty-eight showed gross evidence of erosion. Erosion was differentiated from dentifrice abrasion by its position and from caries by the quality of its base.[10]

Analytic:

Stimulated saliva was collected in 100 c.c. quantities and refrigerated until analyzed. In no instance did more than three days elapse between collection and analysis, as a salivary sample that had been refrigerated for over one year showed no traces of citrate. To determine whether any citrate occurred in saliva, 100-c.c. samples from twenty-five individuals without erosion were tested, qualitatively. The pentabromacetone test of Perlman, Larly, and Johnson[8] was used and showed citrate in nineteen of twenty-five saliva specimens.

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*Since presentation of these data, Zipkin (*Science* 106: 343, Oct. 10, 1947) has presented analyses of stimulated salivas from fifteen men having from 0.64 to 2.04 mg. per 100 c.c.

The method of Deysher and Holm[1] was utilized and modified to measure the citrate content of saliva, quantitatively.

If samples are known to contain reducing materials,[3] aliquots containing less than 25 mg. of citrate are treated with 2 c.c. of H_2SO_4 and the total volume is adjusted to 20 c.c. and boiled for a few minutes. After the solutions cool, 3 to 5 c.c. of saturated bromide water are added. Aliquots of these are placed in test tubes and 0.3 c.c. of H_2SO_4 solution (1:1 of 95 per cent H_2SO_4 and water) and 0.2 c.c. of 1 mol. K Br. and 0.1 c.c. of strong $KMnO_4$ are added. Total volumes are adjusted to 5 c.c. and allowed to stand five minutes at room temperature. They are then chilled in an ice bath and excess $KMnO_4$ is decolorized with 3 per cent H_2O_2, care being taken to keep the temperature below 5° C. during this step. Any excess peroxide is removed with $KMnO_4$. Total volumes are then adjusted to 10 c.c., and 13 c.c. of petroleum ether are added. The tubes are stoppered, shaken vigorously, and centrifuged to break up any emulsion that may have formed. Colorimeter test tubes are prepared containing 5 c.c. of water dioxane mixture (1:1) and 5 c.c. of Na_2S solution (4 Gm. of Na_2S, nonhydrate, per 100 c.c.). Portions of petroleum-ether extract containing pentabromacetone are added. The tubes are shaken vigorously and centrifuged. A light yellow color should be fully developed in five minutes and is stable for several hours. The absorption is determined in a photoelectric colorimeter at 450 millimicrons. A tube containing no citrate which has gone through the same procedure is used as the 100 per cent transmission standard and at least two known samples of citrate should be run with each set of analyses. The color follows Beer's law and the standards are used to calibrate K in the equation, $\log T = KC$. If too large a sample of citrate has been used, a second smaller aliquot of petroleum ether may be taken, avoiding another complete analysis.

RESULTS

In a group of 1,345 young male adults, twenty-eight individuals (2 per cent) showed gross evidence of erosion.

Salivary samples of 100 c.c. each, stimulated by paraffin chewing, were collected from twenty-five young individuals without erosion. Analysis showed:

9 samples (36%)—no citrate to trace amounts
4 samples (16%)—0.4 to 1.0 mg. per 100 c.c.
5 samples (20%)—1.0 to 1.35 mg. per 100 c.c.
7 samples (28%)—1.35 to 1.55 mg. per 100 c.c.

Salivary samples of 100 c.c. each, stimulated by paraffin chewing, were collected from fifteen young men with gross evidence of erosion. Analysis showed:

4 samples (26%)—no citrate to trace amounts
2 samples (13%)—0.2 to 0.6 mg. per 100 c.c.
3 samples (20%)—0.6 to 0.9 mg. per 100 c.c.
3 samples (20%)—0.9 to 1.2 mg. per 100 c.c.
3 samples (20%)—1.2 to 1.5 mg. per 100 c.c.

DISCUSSION

No attempt was made to ascertain whether or not erosion was active in the individuals with evidence of erosion. It is well recognized that erosion may pass through periods of activity and quiescence. It would be necessary to make accurate measurements of the area and depth of the areas of erosion at regular intervals if it were to be stated with certainty that the process was either active or passive. In any future study this should be done to remove the question that was left unanswered in this study, that is, were analyses made on saliva from active or inactive cases?

The results of the analyses of saliva from individuals without erosion confirm the observation that citrate is a constituent of saliva. The citrate content appears to be slightly higher than that found by Pucher, Sherman, and Vickery.[9] There was no appreciable difference between the salivas of individuals with and without evidence of erosion. As suggested above, the areas of erosion seen in the teeth may be historical records of a now inactive process, just as the number of cavities in the mouth at a given time is a record of past caries, and biologic tests, such as the salivary lactobacillus count, may show no susceptibility or activity. From this study, in which the activity of erosion was not determined, no definite statement can be made concerning the relation of salivary citrate to activity of erosion.

SUMMARY AND CONCLUSIONS

In an examination of the teeth of 1,345 young men, twenty-eight instances of dental erosion were observed. This indicates a 2 per cent incidence for young men.

No correlation was found between the presence of erosion and citrate content of the saliva, but it was not apparent whether the erosion was active or quiescent. Further studies should be made of the citrate content of saliva in active erosion.

Saliva from twenty-five young adults without erosion and from fifteen young adults with lesions of erosion was analyzed for citrate content. In 36 per cent of the erosion-free individuals and 26 per cent of those with lesions of erosion, no citrate or only trace amounts were present in the saliva. In 64 per cent of those without erosion, 0.4 to 1.55 mg. per 100 c.c. of citrate was found in the saliva samples, and in 73 per cent of those with lesions of erosion, there was 0.2 to 1.5 mg. per 100 c.c. of citrate in the saliva samples.

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